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DEVELOPMENT OF CYATHUS FASCICULARIS, C. STRIATUS, AND CRUCIBULUM VULGARE[1]

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(WITH PLATES I–VI AND THREE FIGURES)

A number of years ago when looking up literature on Gasteromycetes, the writer was impressed by the fact that no researches upon the development of any of the Nidulariaceae had been published since the classic papers of TULASNE (9), SACHS (8), EIDAM (4), BREFELD (1), and DEBARY (2, 3). From time to time, therefore, as suitable stages were found, the materials for these studies have been collected.

Cyathus fascicularis SCHW.[2]

Materials for the study of this form were collected largely in the various commercial greenhouses of Lincoln, Nebraska, where the fungus grows very abundantly during the late winter and early spring upon the wooden flats in which bulbs are planted for forcing. The mycelium is usually well developed upon the flats at the time they are taken from the storage cellars, and the basidiocarps usually mature at about the same time as the flowering of

[1] Contribution from the Department of Botany, University of Nebraska, New Series, no. 32.

[2] Material of this species was sent to Dr. E. A. BURT of the Missouri Botanical Garden for determination. He says: "It is certainly *C. fascicularis* Schw., which differs from the European specimens of *C. olla* Pers. in our herbarium in not having its sporangiales radiately rugose on the side attached to the funiculus. Although this difference is constant in all specimens examined, it is probably too slight for an adequate specific difference." (*C. olla* Pers. = *C. vernicosus* DC.)

the bulbs. By watching these flats, fruit bodies were found in all stages of development.

Materials for morphological studies were fixed in chrom-acetic (Flemming's or Benda's) solutions, dehydrated, cleared in either xylol or bergamot oil, and sectioned in paraffin. The most satisfactory results were obtained with Benda's solution. The basidiocarps were so filled with grit that many fruit bodies were treated with 10 per cent hydrofluoric acid for 48 hours before being dehydrated. The walls of the peridium and peridioles were at best very hard, especially in older basidiocarps, and this, together with the extreme gelatinization of the filaments which takes place during development, made the sectioning very difficult.

CULTURES.—Artificial cultures were first obtained during the early spring of 1914 from material collected at a local greenhouse. The inoculum used was the peridioles from the nearly mature but unopened basidiocarps. The fruit bodies were treated with a 1.5–1000 solution of mercuric chloride for a few minutes; the epiphragm was then removed with sterile tweezers, and the peridioles removed and planted on sterile agar media. Pure cultures were obtained repeatedly in this way, and also by searing the epiphragm with a hot scalpel and then removing the peridioles. An abundant pure white mycelium showing frequent clamp connections developed at once upon any of the ordinary agar media used in laboratories. Fig. 1 shows a culture of this kind which is five days old.

Mycelium in this condition was fixed, stained, and mounted in Venice turpentine. The mycelium branched abundantly, the branches turning at once in the direction of the growth of the main branch, and coming to lie near and parallel to the main branch. The mycelium was very constantly binucleate, and showed abundant clamp connections.

Mycelium from the agar cultures was transferred to sterile loam, old leaves, half rotten wood, etc., in flask cultures. On such media the mycelium made a very vigorous growth, and strong mycelial strands developed (figs. 2, 4–8). Cultures usually dried out before the formation of basidiocarps, and so water was added from time to time. In this way individual cultures were kept growing for several years. When cultures were kept in the light

a fresh crop of fruit bodies usually developed after each watering. No basidiocarps were found upon cultures kept in the dark, no matter how long the cultures were allowed to remain there. The best results were obtained by keeping the cultures in the dark for three or four months (or longer), until the culture medium seemed thoroughly permeated with mycelium, and then adding water just before placing them in the light, where fruit bodies developed in a few weeks. The most abundant development of basidiocarps

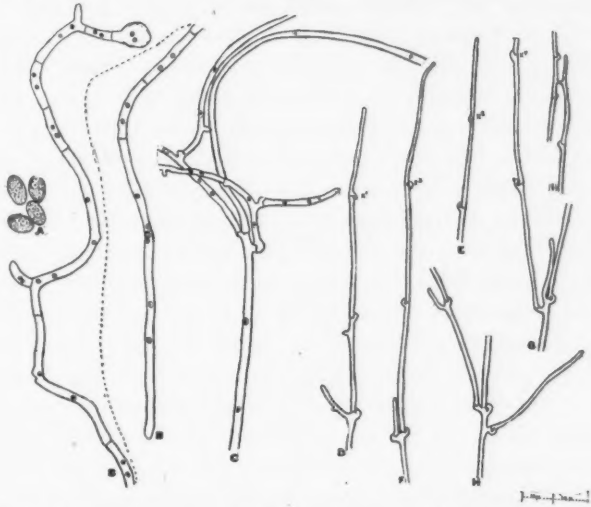

FIG. 1.—*A*, mature spores; *B*, germination spores; *C*, branching and nuclear conditions in older hypha; *D–H*, development of clamp connections (x^1–x^4) and relation of clamps to branching; *I*, anastomosing hyphae.

occurred in cultures exposed in an east window, where direct sunlight reached them during a few hours daily.

HANGING-DROP CULTURES.—Hanging-drop cultures of spores were made repeatedly, but usually spore germination did not take place for some reason. In a few cases, however, especially in one case in which spores from basidiocarps developed in pure cultures were used, abundant germinations in water were obtained. The germ tube usually came from the end of the spore and remained unbranched for some time. In a few cases branching very near

the spore or several germ tubes emerging from the spore were observed. The germinating spores were attached to the cover glass by allowing the water to evaporate until practically dry. They were then fixed in Flemming's fluid, dehydrated, and stained. The spores were constantly binucleate (text fig. 1*A*), and the mycelium developing from them was also composed of binucleate segments or segments which contained paired nuclei (text fig. 1*B*).

Small fragments of the tissue of the peridioles also developed mycelium in the cultures which grew much more extensively. Text fig. 1*C–I* shows the characteristics of the mycelium. In text fig. 1*C*, as well as in most of the mounts obtained where the protoplasmic structure could be determined, the mount was not dried sufficiently to make the mycelium adhere perfectly to the cover glass, so that the direction of growth does not show characteristically. Here, also, there is a binucleate mycelium provided with abundant clamp connections. The clamps appear quite constantly in connection with the branching of the main filaments as well as between the branches. Text fig. 1*D–I* shows in outline portions of a number of such filaments. Text fig. 1*D–G* illustrates the development of the clamps. Near the tip a branch turns backward just above the septum (x^1) and becomes united to the wall just below the septum (x^2). The intervening wall is absorbed (x^3), and a new wall is formed between the clamp and the apical cell from which it arose (x^4). So far as I could determine, there was no evidence that the formation of the clamps was associated constantly with nuclear divisions, as has been described by KNIEP (6) for certain Basidiomycetes. No mounts could be secured, however, in which both walls and nuclei showed distinctly enough in the same specimen to make satisfactory observations on this point. Hyphal anastomoses also occur occasionally (text fig. 1*I*).

MYCELIAL STRANDS.—As soon as growth in culture has reached a few centimeters, the filaments begin to show a tendency to coalesce to form mycelial strands, even upon agar media (fig. 3) and much more conspicuously upon loam, etc. (figs. 4–8). As development continues these strands enlarge until in some cases they become 0.5 mm. in diameter, as found in nature, but usually being 40–200 μ in diameter, the size seemingly being dependent upon the

available nutrition. Figs. 19 and 20 show transverse and longitudinal sections of strands. They are made up of many filaments lying almost parallel to each other and surrounded by a few loosely associated branches which form a vague outer sheath. The cells are very long and 2–5 μ in diameter, the larger being in the center.

ORIGIN OF BASIDIOCARPS.—The basidiocarps originate by the slight differentiation and enlargement of terminal portions of the mycelial strands (figs. 9, 10), and are first discernible in cultures as minute white knots on the mycelium (figs. 5, 6). The filaments of the strand branch in a fanlike manner at the base of the knot (fig. 10), and on the interior above become more slender (about 2 μ in diameter), much branched, and intricately interwoven, making a marked contrast with the filaments of the strand, which are larger and little branched. On the outer tip of the knot, however, is a tuft of hairs the same size as the cells of the mycelial strand. It seems evident, from a study of many slides, that the knot originates slightly below the tip of the mycelial strand, that these terminal hairs are the ends of the filaments which formerly made up the tip of the strand, and that the densely interwoven part just below represents the actual primordium of the basidiocarp. This closely interwoven part is very compact toward the lower part and more open toward the top, where large intermycelial spaces appear.

EARLY INTERNAL DIFFERENTIATION.—The first trace of internal differentiation is the gelatinization of a definite zone of tissue just below the part of the knot which shows the large intermycelial spaces. It extends downward toward the base of the knot in the form of an inverted dome (fig. 11). Fig. 24, between x–x, shows a higher magnification of this zone. The tissue just to the inside of this zone is made up of small, very densely interwoven filaments, and forms the line of demarcation between the primordium of the gleba and that of the peridium. The glebal primordium is composed of filaments of the same size and general character, but more loosely interwoven, so that large intermycelial spaces remain similar to those found in the earliest stages. Toward the top the filaments become more and more loosely interwoven, and pass

gradually into the large, long, flexuous hairs clothing the tip of the young basidiocarp. Fig. 14 shows a slightly older fruit body in which the gelatinization has progressed much farther, and in which the glebal portion shows much more numerous intermycelial spaces.

ORIGIN AND DEVELOPMENT OF PERIDIOLES.—The peridioles originate in the peripheral portions of the glebal region. They are distinguishable as spots where ends of filaments from all sides converge round a common point, as seen in fig. 12, and more highly magnified in fig. 25 in the region of intersection of lines from *a* to *a*. This mode of origin has also been described by FRIES (5) for *Nidularia*. The convergence first takes place in a semicircular manner, extending from the peridium inward toward the central part (fig. 25), but very soon the circle is completed around a common point of convergence. This region is surrounded by a zone of closely interwoven filaments such as originally made up the entire glebal region. A less dense zone with many intermycelial spaces soon tends to form a definite layer within the interlacing filaments, surrounding the converging filaments (except on the under side toward the peridium), and a slightly denser tissue is seen to the outside, which passes into the entirely undifferentiated ground tissue. These faintly marked zones represent the primordia of the walls of the peridiole, while the more or less parallel filaments which interrupt these zones on the under side, toward the peridium, represent the primordium of the funiculus.

The first peridiole to be differentiated is always near the base of the glebal tissue. Other peridioles follow successively from the base of the gleba upward, originating in the same manner from parts of the glebal primordium near its outer part. In the meantime the glebal region has elongated greatly (fig. 13), new growth taking place in an annular region near the apex of the fruit body, where the primordium of the gleba merges with that of the peridium. Fig. 16 shows a higher magnification of a part of the section seen in fig. 13. All the region just described can clearly be differentiated here. The densely interwoven filaments on the outside of the glebal region mark the inner border of the peridium, as will be described later.

As the development of the peridioles progresses, the circle of converging filaments enlarges, due to the multiplication of their elements, largely at the sides, and a central space is observed. This is well shown in figs. 26 and 28, which are higher magnifications of the peridioles in upper and lower portions of the basidiocarp seen in fig. 13. The central space is filled with slime, probably due to the gelatinization of filaments of the fundamental tissue, which are caught in the midst of the more actively growing converging filaments, as in older peridioles remnants of such filaments are clearly present (fig. 29). The cavity in the interior soon takes on an oval or bean shape (figs. 15, 17, 23). This is due to the earlier thickening of the walls of the peridioles on the upper and lower parts, while the sides (ends) remain thinner. Growth remains more active on the sides, and the addition of new elements here, together with mutual pressure, determines the final shape. The enlargement of the cavity in this way continues until the peridiole has reached its mature size.

The converging filaments lining the cavity are at first entirely undifferentiated, appearing in every way similar to the ends of actively growing vegetative filaments such as develop in culture media (figs. 26, 28). Soon they begin to enlarge on the end, and by the time the cavity has reached one-fourth to one-half its ultimate size a definite palisade layer, composed of filaments with enlarged ends, is formed (fig. 29). The palisade layer does not have a uniform even surface as in most of Basidiomycetes, but is made up of basidia and paraphyses of varying lengths. The cells forming the palisade layer are binucleate at first, but the nuclei soon fuse to form the primary nuclei of the basidia and the uninucleate paraphyses (figs. 27, 31). At maturity the entire cavity of the peridiole is densely filled with the oval, constantly binucleate spores (fig. 32).

For some time the walls of the peridioles remain only slightly differentiated (figs. 13, 16). In the subhymenium large intermyceliar spaces remain, and to the outside of this is a denser tissue which will develop into the thick inner wall of the peridiole. Soon, however, the ground tissue of the gleba begins to gelatinize in zones surrounding the already differentiated inner walls of the

peridioles. Figs. 15 and 18 show a basidiocarp where this gelatinization has only taken place around the lower peridioles, while figs. 17 and 21 show a slightly older stage. The tissue remaining between these gelatinizing regions and the denser filaments of the wall of the peridiole becomes the thin, colorless, outer wall of the peridiole. The filaments in this region gelatinize somewhat, but otherwise undergo little change. As the peridioles enlarge, this outer wall becomes stretched out (fig. 23) and remains as a colorless, delicate, easily removable coating over the surface of the mature peridiole. Text fig. 2*a* shows the structure of this layer.

FIG. 2.—Somewhat diagrammatic drawing of wall of mature peridiole through thinner region near side: *a*, outer wall; *b*, pseudoparenchymatic portion of inner wall; *c*, loosely interwoven portion of inner wall; *d*, hymenium.

The cells making up the extreme outer portion of the inner wall of the peridiole become much thickened, pseudoparenchymatic, and brown (fig. 23, text fig. 2*b*), and, showing through the outer hyaline wall, give the characteristic color to the mature peridiole. Within this layer of dark cells is a layer of compactly interwoven filaments whose walls are somewhat gelatinized. This gradually becomes looser as the hymenium is approached (fig. 27, text fig. 2*c*).

In some pure cultures basidiocarps arose between the culture medium and the glass (fig. 4). In such fruit bodies no peridium developed on the side next to the glass, and the whole development of the interior could be followed with a hand lens in an individual basidiocarp. Fig. 6 is an enlargement of the fruit body shown in fig. 4.

DEVELOPMENT OF FUNICULUS.—The funiculus has its origin in the somewhat parallel filaments extending from the innermost surface of the peridium to the primordium of the peridiole. This

appears as a darker area (fig. 25). Later, just to the outside of the inner wall of the peridiole, there appears, between the peridiole and the peridium, a region of actively growing filaments which take the stain readily (fig. 13; higher magnification of left hand peridiole, fig. 28). The filaments in this region elongate rapidly, and soon form a bundle of parallel filaments, as shown in fig. 30, which is a higher magnification of the lower left hand peridiole seen in figs. 15 and 18. Surrounding this bundle are the somewhat gelatinized filaments of the ground tissue, which will form the outer covering of the mature funiculus. The filaments which make up the central bundle of the funiculus continue very active growth, and, being confined by the gelatinizing filaments of the ground tissue, become coiled irregularly in this part. The filaments of the central cord always remain more or less parallel, even in the mature funiculus.

In the mature funiculus (fig. 23) the central cord is attached to the peridiole by the parallel filaments which marked its origin. It immediately twists to form the central coil of the funiculus, and from this passes abruptly at the base into a region of more delicate filaments which merge with the now gelatinizing tissue on the inner wall of the peridium. This central strand is surrounded by the gelatinizing filaments of the ground tissue which constitute its sheath.

The attachment of the funiculus to the peridium is always very weak in this species, so weak, in fact, that as one examines older fruits casually it is not evident, because the peridioles do not seem to be attached. An examination of the peridiole itself, however, shows that the well developed funiculus is always present.

DEVELOPMENT OF PERIDIUM AND EPIPHRAGM.—The mature peridium (fig. 33) is made up of three definite regions, a loosely interwoven outer layer composed of largely longitudinal filaments giving rise to hairs on the surface (*a*), a compact pseudoparenchymatic layer just within this (*b*), and a layer of loosely interwoven, more or less gelatinized filaments which extend from the pseudoparenchymatic layer to the glebal region (*c*).

In the development of the fruit body one can easily trace the origin of these layers. The differentiation takes place first at

the base, as can be observed in sections, and new growth takes place in an apical peripheral zone. This is also clearly shown in text fig. 3*A–D*, where two basidiocarps were marked with India ink, and the position of the lines observed on the older fruit bodies. The outer zone of the peridium comes from the loosely interwoven tissue on the outside of the young knots, which has undergone little differentiation from the condition in the mycelial strand (figs. 11, 12, 17), the zone of pseudoparenchyma, from the closely interwoven filaments within this; while the inner layer is made up of more or less gelatinized filaments which appear very differently at different stages in the development of the basidiocarp. The first differentiation seen in the fruit body is the beginning of gelatinization of the filaments of this inner zone, and in the younger basidiocarps it constitutes the great bulk of the tissue (figs. 13, 15, 17). As the fruit body matures, the continued enlargement of the glebal region causes this inner zone to become more and more compressed, until in a mature specimen it is seen as a zone no wider than the middle zone (fig. 23). At all times the inner zone shows three definite regions so distinctly that they might almost be considered as separate zones. Next to the middle pseudoparenchymatic zone the filaments are loosely interwoven, little if at all gelatinized, and lie longitudinally, following the outline of the middle zone. To the inside these give rise to the wide band of much gelatinized filaments which extend centripetally upward, and merge into the interwoven filaments which border the gleba.

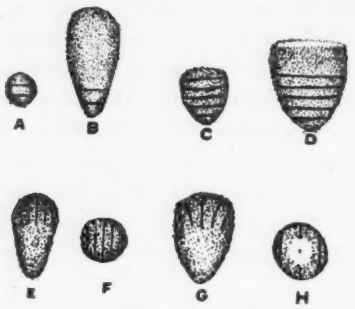

FIG. 3.—*A*, basidiocarp marked with India ink when 3 mm. high to determine region of growth; *B*, same 10 mm. high; *C*, basidiocarp marked when 5 mm. high; *D*, position of marks at time of expansion; *E*, *F*, side and end views of basidiocarp marked just before expansion; *G*, *H*, same basidiocarp with peridium breaking away and epiphragm showing in center.

The peridium covers all of the basidiocarp except the top. To the inside of the abrupt angle shown at the top of the fruit

body (figs. 17, 22) the middle or pseudoparenchymatic layer terminates, and the peridium over the top is composed of the filaments from the outer zone, which here give rise to especially long hairs, and of ungelatinized portions of the inner zone of the peridium.

As the fruit body matures lateral expansion takes place. This causes a rupture in this upper region, the parts structurally connected with the peridium being pulled off from over the top, leaving exposed the little differentiated ground tissue below (figs. 7, 8), which appears as a smooth uniform covering. Text fig. 3*E–H* illustrates how this takes place. A young basidiocarp of about the same age as the one shown in fig. 17 was marked with India ink as illustrated in *E* and *F*, while *G* and *H* show the condition a few days later.

Since the gelatinization of the ground tissue of the gleba begins at the base and progresses upward, the tissue at the top is the last to undergo gelatinization. This, together with the drying effect of the air, results in the formation of a thin membrane, the epiphragm, covering the top, which is exposed by the breaking away of the earlier covering parts. The epiphragm finally ruptures (fig. 5) and itself undergoes gelatinization, leaving the fruit body entirely open on the top. The moisture resulting from gelatinization dries out, and the peridioles sink to the bottom. Thus the basidiocarp assumes its characteristic mature appearance.

Cyathus striatus WILLD.

My studies of this species, based largely upon a small collection of only about 20 basidiocarps made during July 1913 on the campus of the University of Wisconsin at Madison, are necessarily quite incomplete. While this species is quite common in all regions where the writer has collected, it has been impossible to get young stages in most cases. It is included here because it displays some variations in development from that of *C. fascicularis*, and because of the close agreement with TULASNE's (9) work upon this species, and because TULASNE shows its close resemblance structurally to *C. fascicularis*.

The material was fixed in Flemming's medium and weak solutions, dehydrated, cleared in xylol, and sectioned in paraffin.

Cultures of *C. striatus* were made at various times, from material collected near Lincoln, Nebraska, by the same method as used for *C. fascicularis*. An abundant development of mycelium resulted, which was structurally much like that of *C. fascicularis*. The mycelium was at first white, but soon became a dirty brownish color, with many strong mycelial strands. In a few cases small knots appeared upon the mycelium, but mature basidiocarps were never developed.

YOUNG BASIDIOCARPS.—The youngest fruit bodies obtained were 2.5–4 mm. high, and the differentiation had advanced to a considerable degree, so that the peridial and glebal regions were well defined. Figs. 34 and 35 show very low and figs. 37 and 38 higher magnifications of fruit bodies of this type. They show about the same condition as is shown by TULASNE (**9**, *pl. 3, fig. 5*). The peridium has the same structure as in the mature basidiocarp (see fig. 48, the outside to the right), but it is not quite so much hardened. It is made up of three definite zones. The outer of these is made up of loosely interwoven, generally longitudinal filaments, which give rise on the outside to the dense covering of large, stiff, septate hairs about 6–10 μ in diameter; the middle is a pseudoparenchymatic layer which is much wider than the corresponding zone in *C. fascicularis;* while the broad inner layer is composed of more or less gelatinized filaments extending to the central top-shaped primordium of the gleba. This inner zone shows the three definite regions as described for *C. fascicularis*.

At the upper portion of the fruit body is an annular, deeper staining region where the peridial and glebal portions meet (figs. 37–40). This is the region of greatest growth, where new elements are added, both to peridium and gleba, during the elongation of the basidiocarp, just as in *C. fascicularis*. The top of the basidiocarp is covered densely with coarse hairs. The filaments from which these arise are much smaller and lie almost parallel to each other, thus forming a very definite zone over the upper portion of the gleba. The glebal region consists of closely interwoven filaments about 2 μ in diameter, resembling that of *C. fascicularis*.

ORIGIN AND DEVELOPMENT OF PERIDIOLES AND FUNICULUS.—The section shown in figs. 36 and 39 are of a basidiocarp 5 mm.

high when the peridioles are just appearing (cf. TULASNE 9, *pl. 3, fig. 6*). Their mode of origin is entirely like that of *C. fascicularis* as is shown in fig. 44, a higher magnification of the peridiole seen at the left near the base in fig. 39. The only variation in development is that the peridioles seem to appear simultaneously all through the glebal region. That they show in the photograph at the base is due to chance, for in examining the series it is clearly seen that they are equally distributed in all parts. As differentiation continues, we find the individual peridioles passing through identically the same stages as described for *C. fascicularis*, but while in that species the first peridiole differentiated was at the base and remained somewhat in advance of the others throughout its development, in *C. striatus* the upper peridioles soon outstrip the lower ones, so that the upper ones have formed a definite palisade layer before the lower ones have developed more than a small cavity in the center (figs. 40, 41). The same is true at the time of spore formation, the upper peridioles forming spores abundantly, while the lower ones still show a palisade condition. The gelatinization around the peridioles also takes place from above downward.

The structure of the mature peridiole differs from that of *C. fascicularis* only in its general proportions. All layers are much thinner, but especially is this true of the inner wall of the peridiole, which is often no more than 18 μ thick. Most of the apparent thickness of the wall in fig. 46 is due to the long stalks of the basidia which are shown in fig. 45. At maturity the interior of the peridiole is completely filled with the long, slender, binucleate spores characteristic of this species (fig. 47).

The funiculus also shows much the same course of development as in *C. fascicularis*. In the mature funiculus the filaments making up the coil become much more elongated, and therefore more twisted, and the strands forming the attachment to the peridial wall are more strongly developed. Figs. 41, 43, and 46 show stages in its development.

STRIATIONS ON WALL OF PERIDIUM.—The presence of the striations on the inner portion of the upper part of the peridium of the mature fruit body presents an additional point of interest. As the fruit body reaches maturity, the three layers of the peridial

wall extend practically across the top of the basidiocarp, quite in contrast with the condition in *C. fascicularis*. In this upper portion the peridium becomes folded, as seen in a somewhat tangential longitudinal section (fig. 42). At this time the peridioles reach practically to the top of the fruit body, as in younger stages (fig. 40). At the time of expansion this folded upper portion expands, forming the striate projection above the peridioles, which sink to the base of the fruit body as the gelatinized parts dry out.

Crucibulum vulgare TUL.

As *Crucibulum vulgare* is widely distributed and has been more extensively studied than other species of the Nidulariaceae, the writer was especially interested in obtaining it. The material used for most of my studies was discovered by Miss GERTRUDE E. DOUGLAS on an old gunny sack in the woods on the north shore of Beebe Lake on the Cornell University campus while we were collecting together in July, 1916. The old gunny sack was almost covered with fruit bodies in all stages of development. Knowing that I was especially interested in the group, she kindly turned the collection over to me for fixation and study. Material from this was fixed in Benda's solution, dehydrated, cleared in cedar oil, and imbedded in paraffin. Later more material was collected from an old board fence, also on the Cornell campus, and fixed in chrom-acetic solution. The fixation with this solution was not satisfactory, and little use was made of this collection except for purposes of comparison.

Artificial cultures were made of this fungus also, using the same methods as employed for *Cyathus fascicularis* and *C. striatus*. It made a vigorous growth upon all culture media tried. The mycelium was at first white, but soon became a dirty yellow and in parts quite brownish. Flask cultures, such as proved successful for *C. fascicularis*, were tried, but no mature fruit bodies were obtained. The mycelium showed much the same characteristics as that of *C. fascicularis*. Numerous knots appeared on the mycelial strands and on dense mats of mycelium from time to time, but never matured. MOLLIARD (7), however, obtained mature basidiocarps of *Crucibulum vulgare* in similar pure cultures after two and

a half years. The writer's cultures were never maintained for so long a period.

ORIGIN OF BASIDIOCARP.—The basidiocarps arose from mycelial strands or from densely interwoven vegetative mats of hyphae. Not uncommonly young fruit bodies developed on the inside of old peridia which had lost their peridioles. The youngest basidiocarps obtained were about 0.5 mm. in height and showed no internal differentiation, except that the mass took the stain slightly more deeply at the top where the filaments were much smaller (about 1.5 μ in diameter) and more closely interwoven than at the base. Figs. 49–52 show some of these young basidiocarps, while fig. 65 is a higher magnification of the upper portion of the fruit body shown in fig. 50. At the base of the knot the filaments are larger (about 2.5–3 μ in diameter), where they spring from a mycelial strand or mat, and branch at first in a fanlike manner, soon becoming much branched and interwoven as they ascend, and gradually passing into the smaller filaments at the top. In the region made up of these small, closely interwoven filaments many large intermycelial spaces are present (fig. 65). Even these youngest fruit bodies are covered densely with yellow, much branched, thick-walled hairs with toothlike projections (fig. 65). These hairs are 3.5–4 μ in diameter, and so conspicuous that the smallest basidiocarps found could be distinguished only by the presence of yellow tufts made up of these hairs.

INTERNAL DIFFERENTIATION OF BASIDIOCARPS.—The first differentiation here, as in *Cyathus fascicularis*, consists in the gelatinization of filaments. This zone is very vague in its early stages, and it is difficult to be certain just when it begins. Filaments just beginning to gelatinize seem to take the stain more deeply than ungelatinizing filaments, and soon to lose this power and take the stain less deeply. Using these properties and a very slight difference in appearance as determining factors, it seems that the gelatinization begins at the base of the fruit body (fig. 52) and progresses upward in an annular fashion (fig. 53), the darker regions on the sides representing the region just beginning to gelatinize, and the lighter region below representing the more gelatinized filaments. SACHS (**8**), in his description of the development of

the basidiocarps of *Crucibulum*, also finds that the gelatinization begins at the base and takes place upward in the same manner.

This zone of gelatinization soon becomes a well marked one (fig. 55), extending from the upper peripheral part of the fruit body downward, thus outlining the tissue to the interior, the primordium of the gleba, which has undergone no further differentiation. As the filaments to the outside of the glebal region become more and more gelatinized, they elongate and extend in a downward and outward direction to the outside of the deeply stained filaments bordering the glebal primordium (fig. 55). A pronounced lateral expansion of the basidiocarp results. Fig. 66, a higher magnification of the upper left hand portion of fig. 55, shows the structure of the glebal primordium and the tissues bordering it. This is the condition of the fruit body just before peridiole formation.

Fig. 56 shows a fruit body slightly older than the one shown in fig. 55, with the peridioles just beginning to appear. As in *C. fasciculare* and *C. striatus*, the first trace of peridiole formation is found in the appearance of regions near the outer part of the glebal region, where the filaments point radially toward a common center. Fig. 67 shows a higher magnification of the peridiole primordium seen at the base of the fruit body in fig. 56. These inward pointing filaments soon become surrounded by a layer of densely interwoven filaments, and this layer by a region with many intermycelial spaces, which is only slightly interrupted on the side toward the peridium by filaments which lie more parallel to each other and extend downward (fig. 67) to a deeper staining region just outside the zone with intermycelial spaces. This is the primordium of the funiculus.

The further development of the peridiole takes place much as in *C. fasciculare*, and even in its final differentiation shows only variations in minor details (figs. 56–59, 61–62, 70). The peridioles here, contrary to the observations of SACHS, seem to appear in all parts of the gleba almost simultaneously, as in *C. striatus*, and seem to develop almost uniformly in all parts of the fruit body. The only variation was that the lowest peridiole is quite uniformly smaller and sometimes not so well developed. Fig. 64

shows the interior of a mature peridiole with the cavity almost completely filled with the oval binucleate spores. In thickness and consistency the walls of the peridiole are more like those of *C. fascicularis*, and like it are much thinner on the ends than on the sides.

In *Crucibulum*, however, the filaments of the outer wall remain entirely ungelatinized for a longer time, and this tissue is more definitely separated from the gelatinizing filaments surrounding it by a thin border layer of filaments which soon become entirely gelatinized. The remnants of these gelatinized filaments bordering the peridioles take the red stains (saffranin and fuchsin) deeply, thus giving the very conspicuous border to the outer wall of the peridioles as seen in fig. 70. As can be seen in figs. 58 and 61, much more of the undifferentiated glebal tissue remains and undergoes complete gelatinization than in the species of *Cyathus* studied.

The funiculus has its origin, as already pointed out, in a region of more active growth just to the under side of the young peridiole (figs. 56, 67). In this region the filaments elongate very rapidly, and soon develop into a stout bundle of parallel filaments extending from the young peridiole to the glebal wall (figs. 57, 68). The filaments forming this bundle are surrounded by gelatinizing filaments which extend in the same general direction, and will form the sheath of the funiculus. The central strand of parallel filaments continues its elongation as the basidiocarp develops, and by the time a palisade layer is differentiated (figs. 58, 59) two definite regions are easily distinguishable, a region of much coiled and twisted filaments forming a knot just below the peridiole, and a long slender strand reaching down much below the present position of the peridiole and widening out as it attaches itself to the peridium (fig. 59). This was also described by TULASNE.

These strands are easily traceable far down the sides of the peridium by the conspicuous clamp connections which are abundantly present (fig. 60). The position of the clamps indicates that growth in the strand is upward from the peridial region. Among the gelatinizing filaments surrounding the central strand of the funiculus can clearly be seen the less gelatinized filaments making up the sheath of the funiculus (figs. 61, 62, 70). A funiculus from a nearly mature basidiocarp is shown in figs. 69 and 70.

DEVELOPMENT OF PERIDIUM AND EPIPHRAGM.—The mature peridium (fig. 63) would closely resemble that of *Cyathus fascicularis* and *C. striatus* if the middle pseudoparenchymatic layer were omitted. The outer layer consists of loosely interwoven, largely longitudinal filaments giving rise to long flexuous hairs on the outside. Among these hairs can be seen only rarely, more often toward the top, traces of the much branched, sharp pointed hairs that originally covered the entire fruit body. The filaments comprising the outer part are thick-walled and brownish, becoming thinner-walled and colorless toward the inner portion, and thus merging into the inner layer, which is composed of more or less gelatinized filaments. This peridium covers the sides and converges slightly over the top of unopened fruit bodies (fig. 61).

These zones of the peridium are quite well differentiated before the primordia of the peridioles appear, and the outer zone undergoes little change during subsequent development. On the other hand, the inner zone changes greatly. In the young fruit bodies (figs. 55, 56) it is a wide zone, but as the glebal region develops it becomes more and more compressed (figs. 57, 59), until in the mature basidiocarp it forms an almost indistinguishable layer (figs. 61, 70).

The epiphragm covers the upper surface of the fruit body and is definitely marked by the time the basidiocarp is half developed (fig. 58). It consists of the entirely undifferentiated upper part of the basidiocarp as seen in younger stages (figs. 55, 56), and is as densely covered by the much branched, pointed hairs as was the original young fruit body (figs. 49–54). Following the gelatinization of the filaments surrounding the peridioles, those constituting the epiphragm also undergo gelatinization. The epiphragm just before gelatinization is shown in fig. 62. There is no opening up of the upper portion by the spreading of the superficial layer as described for *C. fascicularis*.

Comparisons

From FRIES'S description of the development of *Nidularia* it evidently has the simplest structure of any of the Nidulariaceae whose development has been studied. Its development seems to correspond very closely with that of *Crucibulum*, except that the

funiculus is entirely lacking. Both are covered at first with toothed hairs, and the internal structures are similar. The zone of gelatinization, which is the first differentiation to be observed, is similar, except that in *Nidularia* it seems to extend over the top of the basidiocarp also. The development in both seems to be basal, as shown by the location of the toothed hairs on the upper portions of mature basidiocarps. The tissues covering the top of the fruit body just before opening are seemingly homologous, but more definitely limited in *Crucibulum*. The structure of the peridium and the origin and development of the peridioles are similar, but in *Nidularia* they appear first at the base, while in *Crucibulum* they appear simultaneously. The structure of the walls of the peridioles, as described by FRIES, is very different seemingly. The close relationship of these forms, however, is very evident.

In *Cyathus* more differences appear, especially in the structure of the peridium and funiculus, but the general type of development is very similar. Of the forms studied, *C. fascicularis* shows closer resemblances to *Crucibulum* than does *C. striatus*. The part of the gleba to become differentiated first and mature first seems to be very variable in the genera and species studied. While in my materials it has seemed to remain quite constant for each species, there is the possibility that it may vary even in the same species.

Summary

1. All three species are easily grown on artificial media. Mature fruit bodies were obtained only in cultures of *Cyathus fascicularis*.

2. The mycelia of all are very similar except for color. Clamp connections are abundantly present, and conspicuous mycelial strands are formed. The cells of *Cyathus fascicularis* are binucleate or composed of segments with paired nuclei.

3. The basidiocarps of *Cyathus fascicularis* and *C. striatus* arise from mycelial strands in all cases observed; while those of *Crucibulum vulgare* may arise from mycelial strands, dense mats of hyphae, or from the interior of old peridia.

4. The primordium of the basidiocarp seems to have its origin slightly below the tip of the strand, and consists of closely interwoven filaments smaller than those of the strand.

5. The first marked internal differentiation in all three consists of the gelatinization of a zone of hyphae in a region that will become a part of the inner wall of the peridium. A zone of closely interwoven filaments just to the inside of this forms the boundary between gleba and peridium.

6. The origin and development of the peridioles is similar in all. Each peridiole originates around a center, toward which the ends of filaments converge. The structure of the walls of the peridioles differs only in relative proportions.

7. The first peridioles to be differentiated in *Cyathus fasciculatis* are toward the base of the gleba, and later other peridioles develop above them. The peridioles at the base mature before those nearer the top of the gleba. In *C. striatus* and *Crucibulum vulgare* the peridioles appear almost simultaneously throughout the glebal region; but the upper peridioles in *C. striatus* mature before the lower ones; while in *C. vulgare* the development is more uniform.

8. The funiculus of *Crucibulum* differs greatly in form from that of *Cyathus*, especially in the length of the strand at the base. The origin is similar in both.

9. The most marked difference between *Crucibulum* and *Cyathus* is in the structure of the walls of the peridia. In *Cyathus* a middle pseudoparenchymatic layer is present which is entirely wanting in *Crucibulum*.

10. During the expansion of the basidiocarp in *Cyathus* the peridium is pulled off from over the glebal region, leaving parts of the ungelatinized ground tissue to form, for a time, a thin covering (the epiphragm). In *Crucibulum* the epiphragm consists of the undifferentiated primordial tissue covered with branched hairs. This undergoes gelatinization at maturity.

11. The spores of all are constantly binucleate.

I wish to express my indebtedness to the late Professor GEORGE F. ATKINSON, who saw a first draft of this paper, for a number of helpful suggestions, to Miss GERTRUDE E. DOUGLAS for the collection of much of the material used in the study of *Crucibulum vulgare*, and to Dr. E. A. BURT for going over the manuscript of

this paper with me and making suggestions, and for the determination of *Cyathus fascicularis*.

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EXPLANATION OF PLATES I–VI

Figs. 1–8 were made with a Century camera and Zeiss-Tessar lens. The photomicrographs were made as follows: figs. 9–23, 34–43, 46, 49–59, 61, 62, with a horizontal Zeiss camera; figs. 19, 20, 24–33, 44, 45, 47, 48, 60, 63, 64, 69, 70, with a Bausch and Lomb vertical camera and Zeiss lenses; and figs. 65–68 with a Bausch and Lomb vertical camera and Leitz lenses.

PLATES I–III

Cyathus fascicularis

FIG. 1.—Culture growing from peridiole, 5 days old; $\times 1\frac{3}{4}$.

FIG. 2.—Young culture growing on wood and loam; $\times \frac{1}{2}$.

FIG. 3.—Tube culture showing beginnings of strand formation; $\times \frac{7}{8}$.

FIG. 4.—Basidiocarps developing between loam and sides of culture jar in which no peridium formed on side next to glass; $\times \frac{1}{2}$.

FIG. 6.—Basidiocarps shown in fig. 4; $\times 2$.

FIGS. 5, 7, 8.—Basidiocarps and mycelial strands formed in cultures, slightly reduced in size.

FIG. 9.—Median longitudinal section of young basidiocarp with mycelial strand from which it developed; ×31.

FIG. 10.—Slightly older basidiocarp, cut somewhat diagonally to show relation of filaments of strand to basidiocarp; ×31.

FIG. 11.—Median longitudinal section of basidiocarp showing origin of inverted gelatinous dome, which is first internal differentiation to take place; ×31.

FIG. 12.—Median longitudinal section of basidiocarp with zone of gelatinization well defined and origin of peridioles evident; ×31.

FIG. 13.—Longitudinal section (median at top, little to one side of center at base) of basidiocarp, with all parts of mature fruit body distinguishable; ×13.

FIG. 14.—Median longitudinal section of basidiocarp intermediate between figs. 11 and 12; ×31.

FIG. 15.—Median longitudinal section of basidiocarp, somewhat older than fig. 13; ×13.

FIG. 16.—Higher magnification of portion of basidiocarp shown in fig. 13; ×31.

FIG. 17.—Median longitudinal section of basidiocarp slightly older than fig. 15; ×13.

FIG. 18.—Higher magnification of portion of section shown in fig. 15; ×31.

FIG. 19.—Transection of mycelial strand; ×225.

FIG. 20.—Longitudinal section of mycelial strand; ×225.

FIG. 21.—Higher magnification of lower peridiole shown in fig. 17; ×31.

FIG. 22.—Higher magnification of upper portion of section shown in fig. 17; ×31.

FIG. 23.—One peridiole, with funiculus and portion of peridium from longitudinal section of basidiocarp; ×31.

FIG. 24.—Higher magnification of gelatinizing zone (x–x) from section shown in fig. 11; ×225.

FIG. 25.—Higher magnification of earliest trace of peridiole differentiation; center of converging filaments at point of intersection of line A–A; ×225.

FIG. 26.—Higher magnification of peridiole from upper portion of fruit body shown in figs. 13 and 16; ×225.

FIG. 27.—Hymenium and subhymenium as seen in peridiole with mature spores; ×225.

FIG. 28.—Higher magnification of peridiole shown at lower left hand side of figs. 13 and 16; ×225.

FIG. 29.—Section of peridiole about one-half mature size, showing structure of hymenium and tissues below it; ×225.

FIG. 30.—Funiculus of peridiole shown in fig. 29; ×225.

FIG. 31.—Much higher magnification of small portion of hymenium from peridiole just beginning to form spores, showing young binucleate basidia and uninucleate basidia; ×585.

FIG. 32.—Mature binucleate spores from same peridiole as fig. 31; ×585.

FIG. 33.—Longitudinal section of peridium of mature basidiocarp: *a*, loose outer layer; *b*, pseudoparenchymatic layer; *c*, inner, loosely interwoven, and more or less gelatinized layer; ×225.

PLATE IV

Cyathus striatus

FIGS. 34, 35.—Median longitudinal sections of youngest basidiocarps found (basidiocarp in fig. 35 bent at base so that section of lateral wall is seen at base); ×14.

FIG. 36.—Median longitudinal section of older basidiocarp with peridioles appearing; ×14.

FIGS. 37–39.—Higher magnifications of basidiocarps shown in figs 34, 35, 36; ×34.

FIG. 40.—Median longitudinal section of still older basidiocarp; ×73.

FIG. 41.—Higher magnification of portion of section shown in fig. 41; ×34.

FIG. 42.—Tangential longitudinal section showing folding of incurved portion of peridium; ×31.

FIG. 43.—Section of nearly mature peridiole; ×34.

FIG. 44.—Higher magnification of portion of section seen in fig. 39 showing converging filaments in early development of peridiole; ×225.

FIG. 45.—Section of peridiole producing spores, showing hymenial layer; ×225.

Fig. 46.—Section of peridiole producing spores and funiculus; ×225.

FIG. 47.—Mature spores; ×360.

FIG. 48.—Section of peridium of mature basidiocarp; ×225.

PLATES V, VI

Crucibulum vulgare

FIGS. 49–52.—Median longitudinal sections of very young basidiocarps; ×31.

FIGS. 53–55.—Median longitudinal sections of basidiocarp showing early stages in gelatinization of filaments bordering primordium of gleba; ×31.

FIG. 56.—Median longitudinal section showing origin of peridioles; ×31.

FIG. 57.—Slightly tangential longitudinal section showing early stage in development of peridiole and funiculus; ×31.

FIG. 58.—Longitudinal section of older basidiocarp; ×13.

FIG. 59.—Section of peridiole before spore formation, and funiculus; ×31.

FIG. 60.—Section of mature funiculus showing clamp connections; ×360.

FIG. 61.—Longitudinal section of nearly mature basidiocarp; ×13.

FIG. 62.—Portion of longitudinal section of older basidiocarp; ×31.

FIG. 63.—Longitudinal section of peridium of mature basidiocarp; ×225.

FIG. 64.—Section of peridiole showing hymenium and interior filled with spores; ×360.

FIG. 65.—Higher magnification of upper portion of basidiocarp shown in fig. 50; ×225.

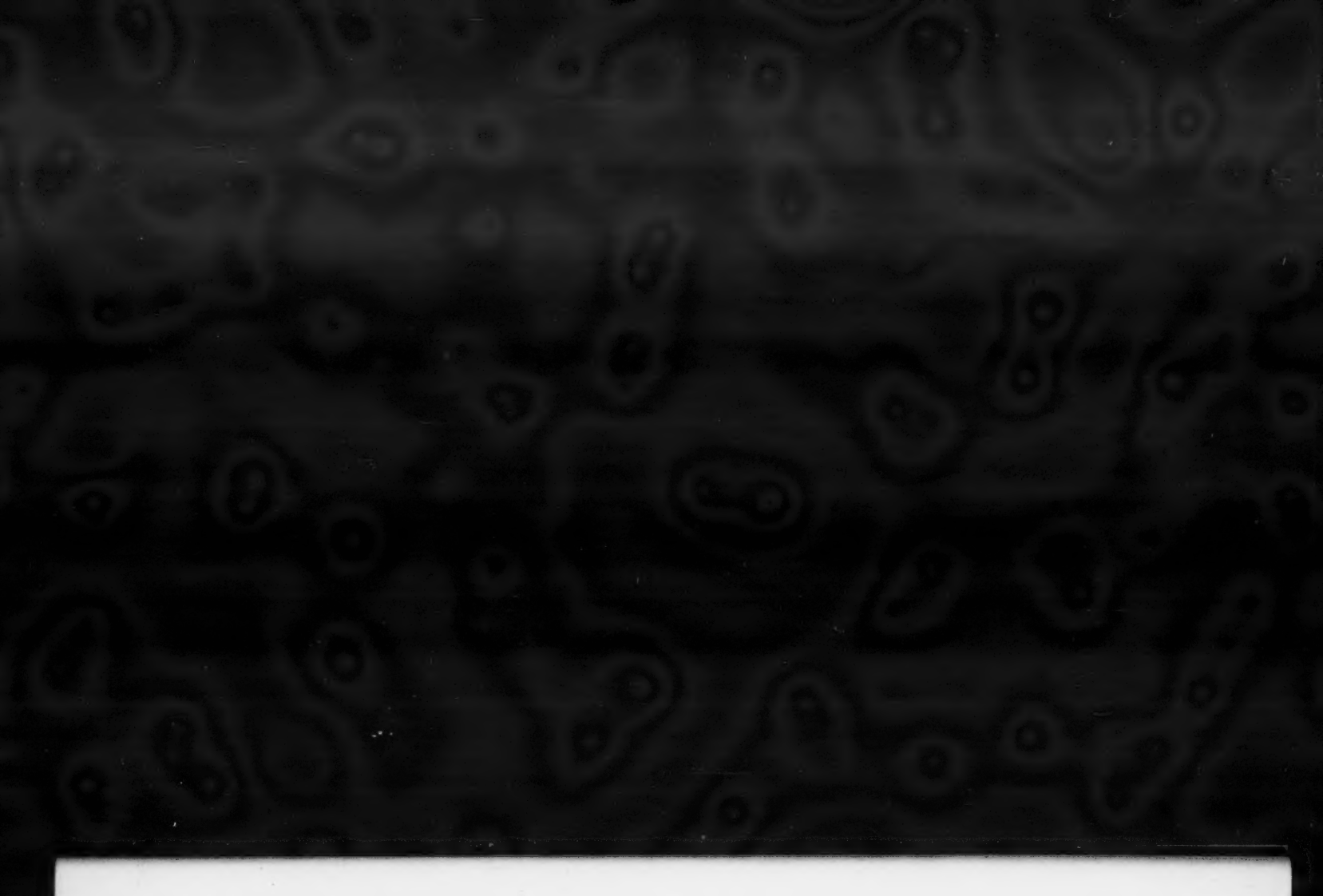
FIG. 66.—Higher magnification of upper left hand portion of fig. 55; ×225.

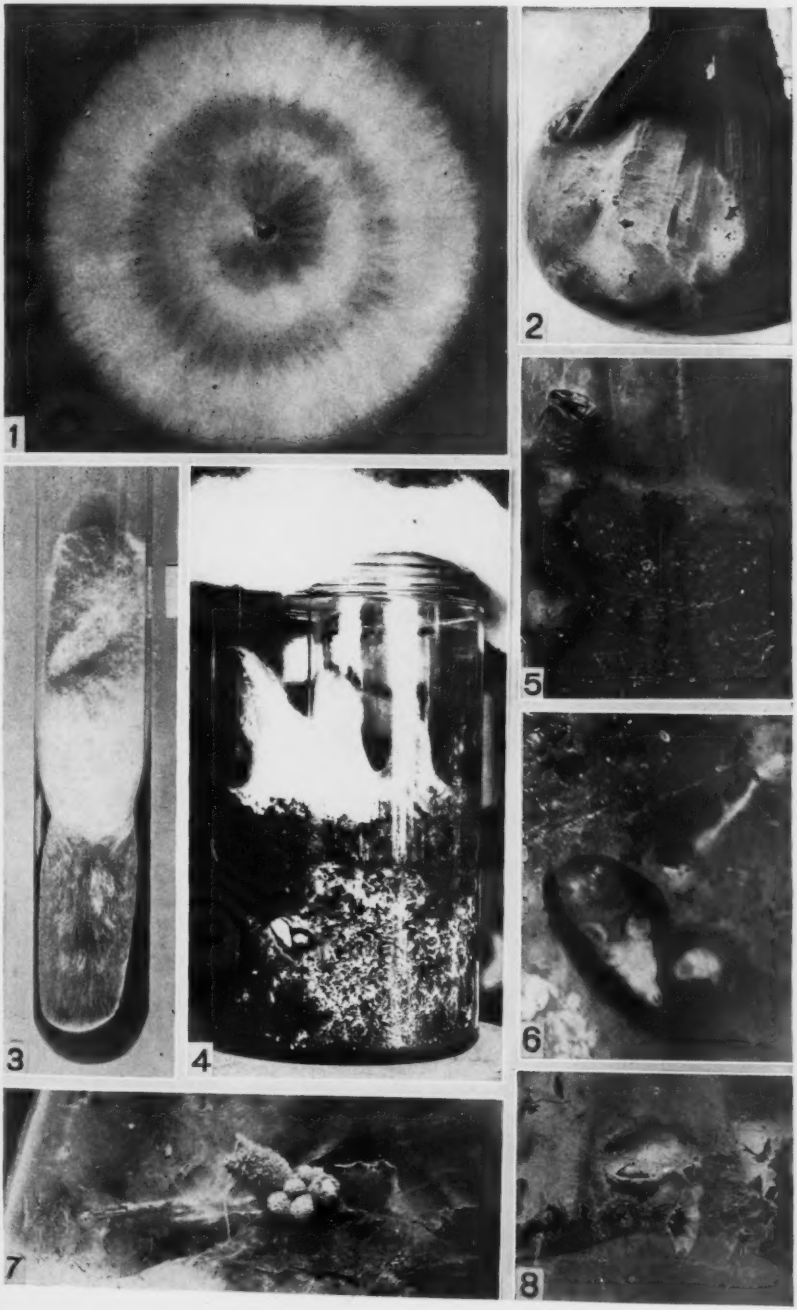
FIG. 67.—Higher magnification of peridiole initial near base of basidiocarp shown in fig. 56; ×225.

FIG. 68.—Higher magnification of funiculus to peridiole shown to left in fig. 57; ×225.

FIG. 69.—Higher magnification of upper portion of funiculus shown in fig. 70; ×360.

FIG. 70.—Funiculus from another section of basidiocarp shown in fig. 61; ×31.





WALKER on CYATHUS

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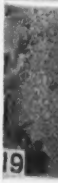

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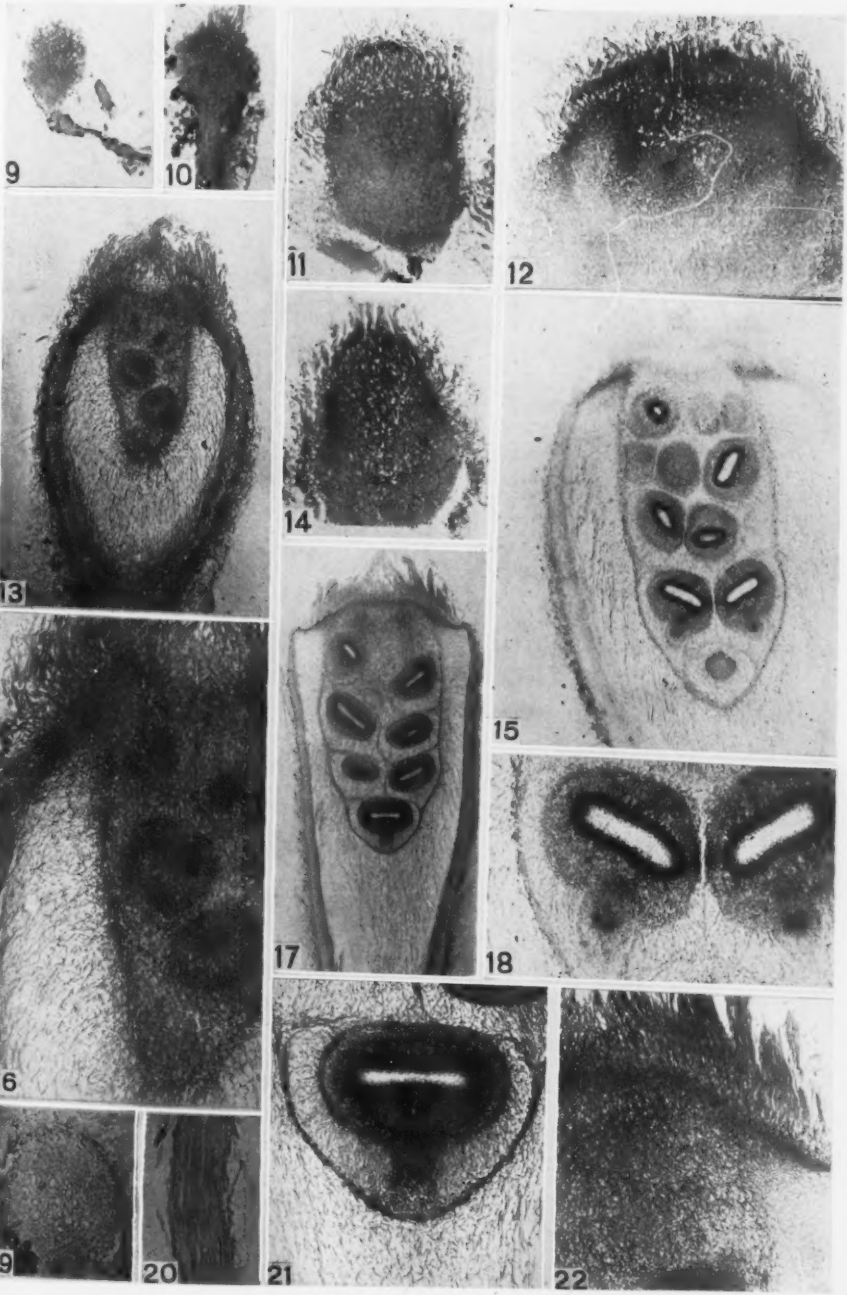

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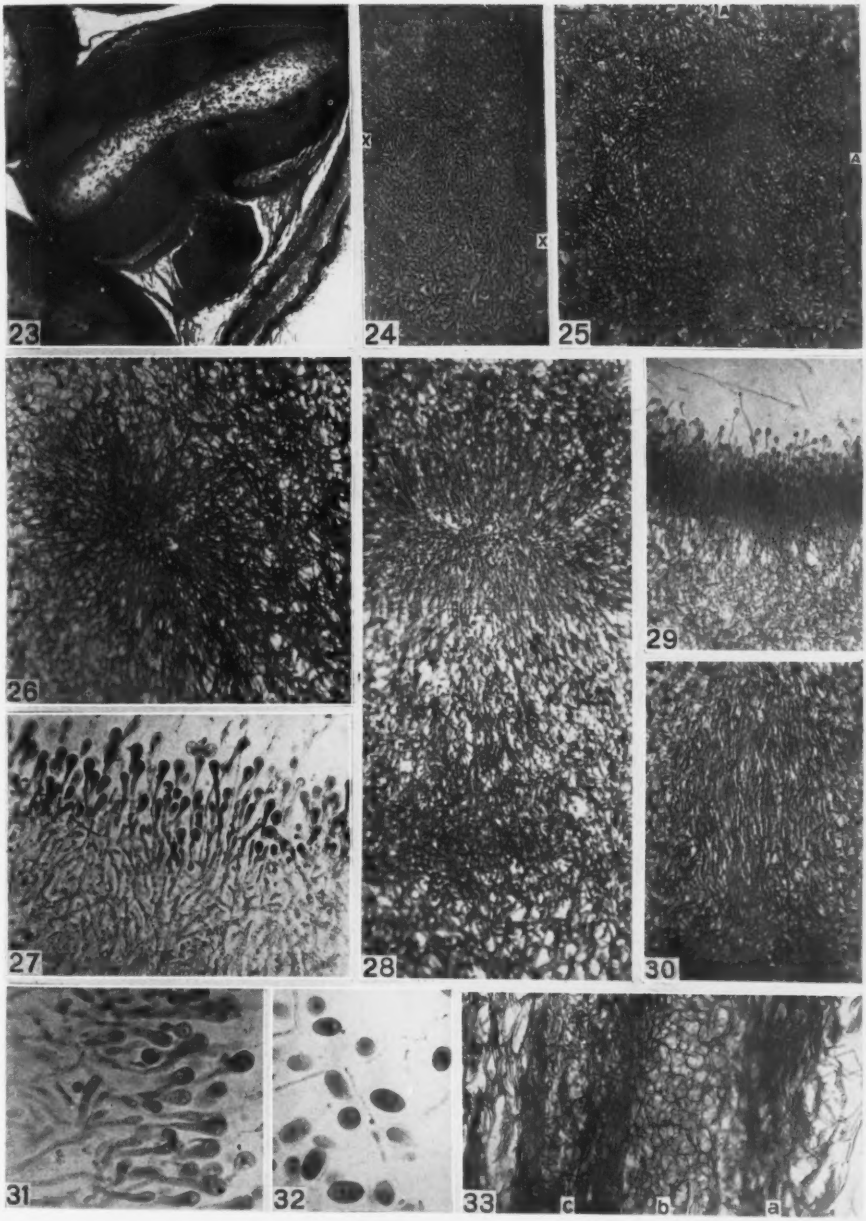

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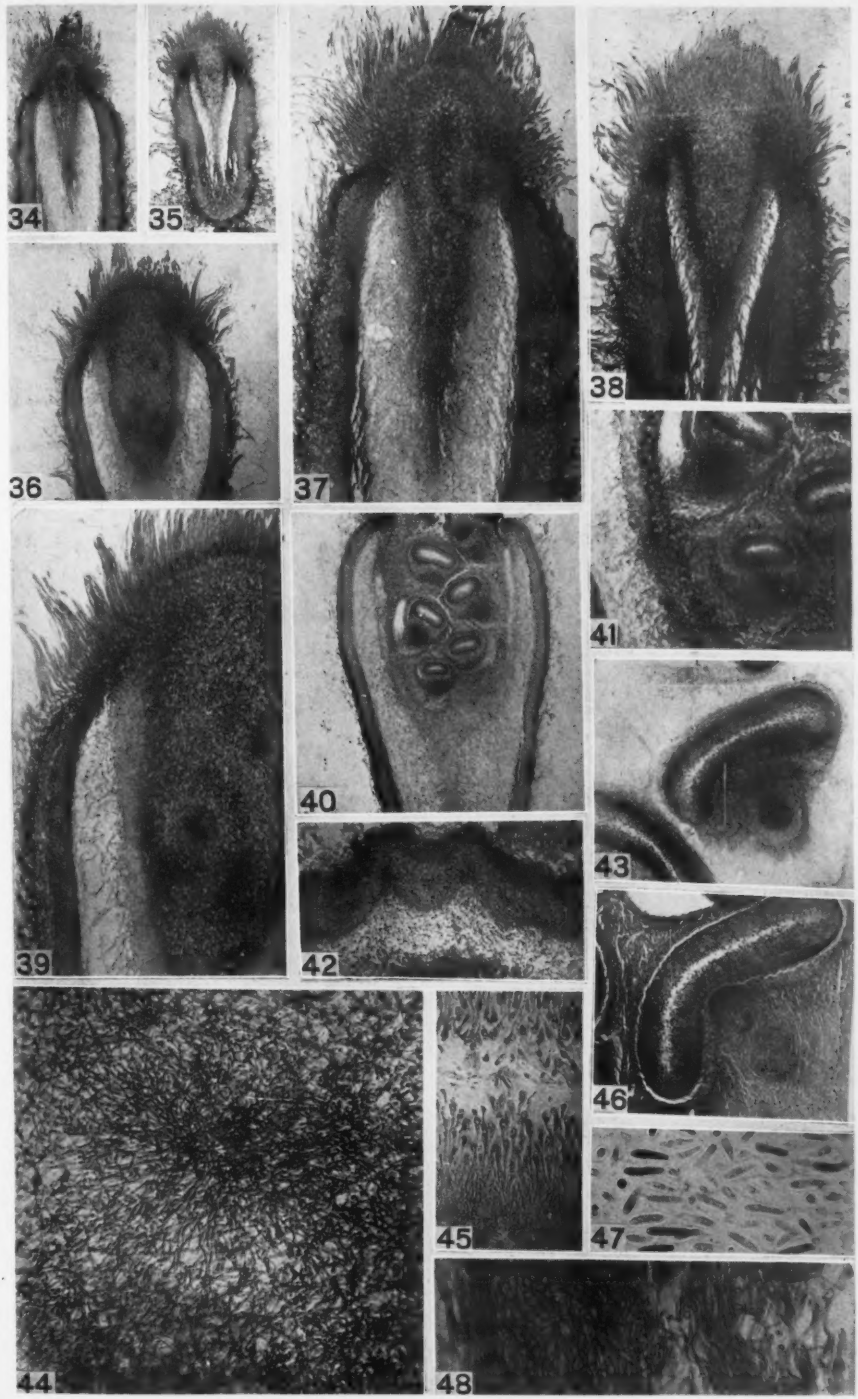

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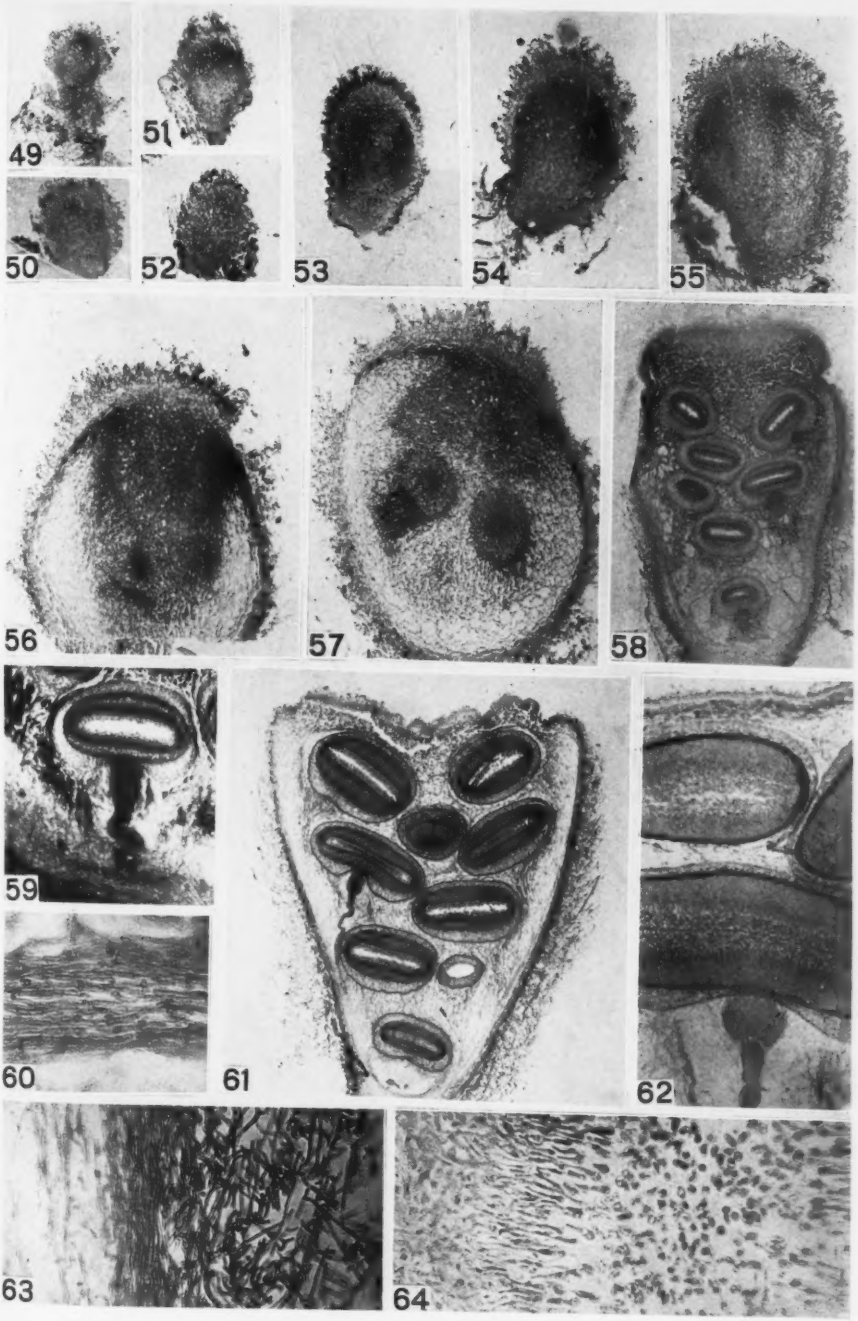

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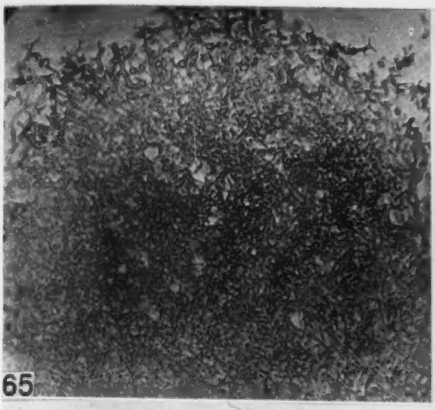
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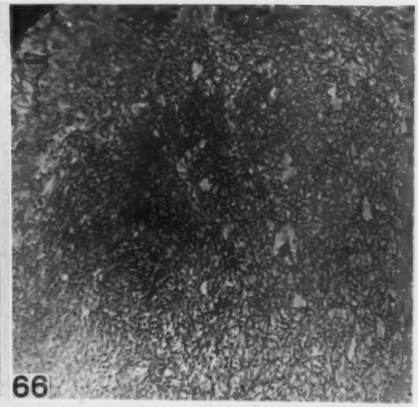
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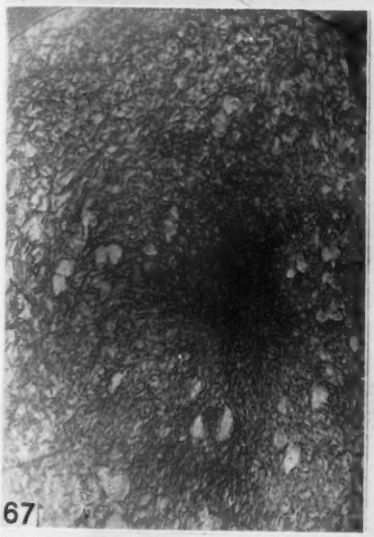
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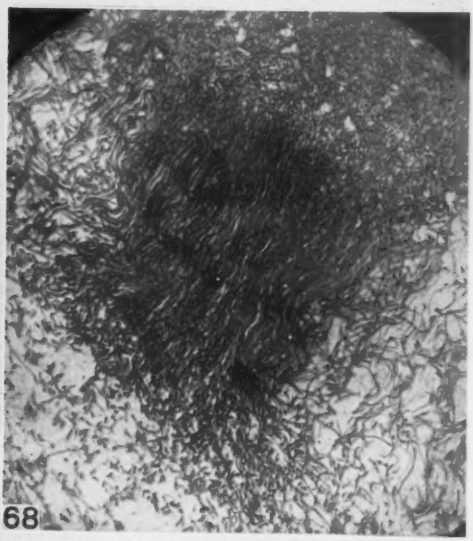
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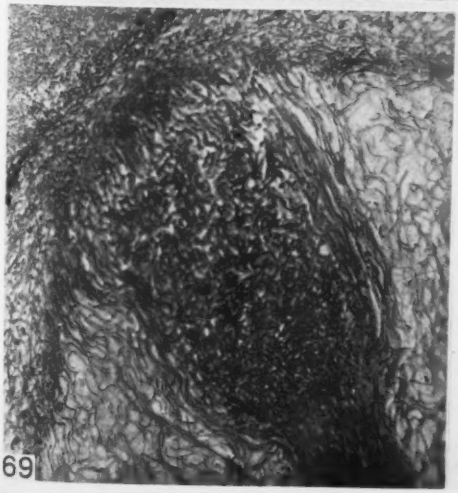
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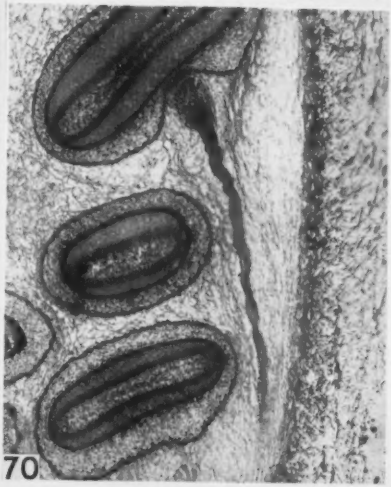
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EFFECT OF UNILATERAL MONOCHROMATIC LIGHT AND GROUP ORIENTATION ON THE POLARITY OF GERMINATING FUCUS SPORES

ANNIE MAY HURD

(WITH TWO FIGURES)

Introduction

The power of light stimuli to produce orientations and tropisms is a phenomenon which has been widely demonstrated in both the plant and animal kingdoms. Not only can unilateral illumination direct movements and growth, but in some species of plants, namely, *Equisetum*, *Fucus*, *Puccinia*, and related forms, natural white light has been found to establish the direction of the first cleavage plane of the germinating spore. Since in such cases the cell on the shaded side of the spore becomes the rhizoidal cell, the polarity of the plant is determined by light, irrespective of gravity. The primary purpose of the present investigation was to determine whether all wave lengths of light, the intensity factor being eliminated, are able to bring about this orientation and establish the polarity of the germinating spores of *Fucus inflatus*. Subsidiary studies which have been made in this connection with interesting results are (1) on that most interesting and little known phenomenon which I have called "group orientation," consisting in the orientation of the cleavage plane and the establishment of the apical and basal ends of the dividing spore by the direction of some other spore or group of spores in close proximity; and (2) on the phototropisms of the young rhizoids in monochromatic lights of equal intensities.

In reviewing the literature on biological experiments with monochromatic light, one is struck by the small number of quantitative records of the quality and especially of the intensity of the illuminations used. The ordinary light filters used to obtain monochromatic light transmit not only those wave lengths which predominate and give the color to the screen, but also other parts

of the spectrum, the presence of which can be detected only by a spectroscopic analysis. For example, certain results are frequently ascribed to blue light, with no record of just what range of the spectrum is used, nor what wave lengths other than the predominating ones are acting.

Another source of inaccuracy has been the neglect or oversight of the great variation in the intensity or quantity of radiant energy transmitted by the color screens. Biological experimenters for the most part have failed to take into consideration the fact that the quantity as well as the quality of the light stimulus varies with the different colors, and that the former variable must be eliminated before results can be attributed to differences in wave length alone. In some cases the importance of differences in the intensity factor has been recognized, but no method was known to the writer whereby the different colored lights could be compared as to their amounts of radiant energy (10).

There have been, of course, several methods devised by means of which the relative intensity of monochromatic lights can be measured. The first investigation in which the attempt was made to get monochromatic light of known wave length and equal energy was that of BLAAUW (1). He used glass color screens, and states that the lights transmitted were equal in intensity when measured with a Weber photometer. The accuracy of this method can be seriously questioned.

The next exact work of this nature was done by KNIEP and MINDER (11). They used a blue and a red color screen, and a green solution, with sunlight as the source of light. The wave lengths to which each was transparent were known; and the energy behind each was determined by means of a thermopile and d'Arsonval galvanometer. The interference of the long heat rays was prevented by inserting a water layer in a parallel-sided container between the thermopile and the source of light.

DAY (5) obtained light of known wave length by means of a spectrum from Nernst glowers, formed by a carbon bisulphide prism and cut down by a diaphragm with narrow vertical slits which could be adjusted so as to allow any desired region of the spectrum to be transmitted. In this adjustment a spectroscope

was used to determine the exact range of wave lengths passing through the slit in each of the four illuminations used (red, yellow, green, and blue). DAY measured the intensity of each with a Boys radiomicrometer, and balanced them by varying the number of glowers employed in the lamps. Thus there was one glower for the red light, two for the yellow, and three for the green and blue.

LAURENS (12), in an investigation of the reactions of amphibians, employed these same methods and the same apparatus for the quantitative analysis of the monochromatic light he used, and balanced them similarly with respect to their relative intensity. GROSS (9) also used the same methods in determining the reactions of arthropods to monochromatic light. MAST (14) measured the different effects of monochromatic light by the orientation of organisms in a field in which two differently colored beams crossed at right angles. He used spectral regions of known energy and wave length. The method of obtaining the distribution of energy is not described. PARR (15) did quantitative work on the response of *Pilobolus* to different wave lengths, using apparatus of the type employed by DAY and LAURENS.

An instrument has been devised by MACDOUGAL and SPOEHR (13) which measures the total radiant energy of any light in terms of its dissociation effect on a photosensitive substance. This is measured by a galvanometer. The advantages in the use of this "photoelectric cell" are said to be its extreme sensitiveness to the wave lengths of the blue end of the spectrum, and the fact that its action in light is "more nearly that of the organism than that of any other light measuring instrument available."

There have been, therefore, three exact methods worked out for biological experiments to obtain a quantitative analysis of light stimuli, namely, those of KNIEP and MINDER, DAY, and MACDOUGAL and SPOEHR. The interesting apparatus devised by PATTEN (16), whereby a quantitative measurement of the reactions of organisms subjected to two beams of light of different intensity is obtained, might be mentioned here. The measurement is in terms of the angular deflections from an initial path of locomotion. The same methods might be applied to work with colored lights.

A quantitative measurement of the greater effectiveness of one spectral region over another of equal intensity might be measured by the angular deviation of the path of a motile organism from a line perpendicular to a line connecting the two sources.

All these methods, with the exception of those of KNIEP and MINDER, involve special apparatus often not easily available. For many problems simpler methods will accomplish the same ends. For the present investigation a method has been devised whereby color screens of known wave length transmission are used, and their relative intensities measured by means of a thermopile and galvanometer, and made equal by adjusting the distances from the light source.

Apparatus and methods

As biological science becomes more exact, with the tendency to reduce the expression of natural phenomena to mathematical formulae, it is obviously essential to define stimuli of all sorts quantitatively. Indefinite or incomplete records of light stimuli can no longer be attributed to the lack of means of measuring them, because access to a spectroscope and thermopile makes it possible to analyze any light qualitatively and quantitatively.

There are two methods of obtaining monochromatic light for biological experiments, namely, the projection of a spectrum upon the organisms, or the use of filtered light passed through a color screen. The former is theoretically the better for exact work, but technical difficulties, such as limited dispersion and low intensity, make it impractical for many investigations. Light filters of glass are the most convenient means of securing approximately monochromatic light when unilateral illumination is desired. Ordinary color screens transmit too wide a range of wave lengths for exact work, and at present there are very few whose light is of sufficient homogeneity. The best is the Wratten filter screen, which consists of a dyed gelatine film between two glass plates. MACDOUGAL and SPOEHR have described some colored glass screens designed by them for biological work, but the range of wave lengths to which each of them is transparent is considerably greater than for some of the Wratten filters.

In the experiments to be described, Wratten light filters were used, each of which was fitted as a window in the end of a dark box. Each transmitted only a narrow range of wave lengths, but together they embraced the whole of the visible spectrum. The wave lengths to which each screen was transparent were determined by testing the light transmitted by each with a direct vision spectroscope with a wave-length scale attached. Thus the quality of the light stimulus acting in each box is accurately known. The dark boxes were 10×13 cm. and 8 cm. high, in one end of each of which a hole was cut so that one of the light filters, 5×5 cm., might be fitted into it. The boxes were made light-tight with tightly fitting covers, and were painted black inside to guard against reflections within the box. The dishes used for the cultures were either the ordinary Petri dishes or special dishes made of microscope slides cemented together with zinc cement so as to make shallow oblong dishes 7.5×2.5 cm. and 1 cm. deep. It was at first deemed necessary to use such flat-sided dishes in order to prevent possible complications from reflected and refracted light in the curving sides of round dishes, but later it was found that the same results were secured in the Petri dishes. In order to expose more than one dish behind each screen so that none would be shaded by another, a rack was made to fit inside the box with cleats projecting inward from the ends so that three dishes could be slipped into it, one above the other. The light, entering through the screen at the end of the box, fell equally on the one exposed side of each of the three dishes. The rack containing the dishes could easily be lifted out and carried to the microscope for examination without disturbing the material under investigation.

The source of light first used with the filters was the electric arc. The advantage of this light over any other artificial light is that it gives all the wave lengths of the visible spectrum, so that all the filters could be used in each exposure, insuring identical conditions of temperature, constancy of illumination, etc. The disadvantages are several. In the first place, the intensity is constantly changing as the carbons burn and the arcs get longer, and the lessening of the intensity may not be the same for all the wave lengths. In the second place, fluctuations in the current

cause large variations in the intensity. In the third place, unless an automatically adjusted arc is available, it is necessary to adjust the carbons by hand every 5 to 15 minutes, and, when an 8 hour illumination is desired, this entails considerable inconvenience. The experiments were begun with this light, however, and the results on rhizoidal phototropisms in monochromatic lights made equal in intensity were obtained with it.

The spectroscopic analysis of the light passing the screens determines definitely the quality of light entering each box. It is at once evident that the quantity or intensity of light behind filters placed at equal distances from the source varies, both because the intensity of light transmitted by the different screens is different, and because the different colors are not radiated by the arc with equal intensity. This being the case, differences in results obtained behind the screens could not be attributed to differences in the quality of the light stimulus alone.

One of the simplest means of comparing the radiant energy of colored lights is by the use of the thermopile and sensitive galvanometer. The thermopile is very sensitive to the energy of any ether vibrations, whether they be the longer infra-red or so-called heat rays, or the shorter actinic rays of the spectrum. The method developed for eliminating the intensity variable in the use of the filters employed for these investigations consisted in finding the distances from the arc at which each box with its colored window should be placed in order that the intensity might be equal in each case. These distances were those at which the deflections of the galvanometer were equal when the thermopile was exposed to the arc screened by each filter in turn.

It seems necessary, on account of the questions which have been raised during the course of this work, to state that the thermopile is equally sensitive to the energy of the red and of the violet ends of the spectrum, and is, therefore, an accurate measure of the total amount of light acting behind each color screen. The difference between heat and light is only a matter of wave length. The thermopile measures light in terms of the electric current produced by the difference in temperature of the exposed and unexposed junctions; but it does so by virtue of the fact that the energy

of whatever vibrations fall upon it, be they long and therefore heating in their physiological effect, or short and therefore perceived as light, is converted into heat energy upon being absorbed by the exposed junction of the thermopile. In other words, the light of the blue end of the spectrum produces an electromotive force much less than that of the infra-red, but no less measurable.

The instruments used in the energy calibration of these screens were a Hilger thermopile with junctions of bismuth and silver, and a moderately sensitive galvanometer (d'Arsonval). An electric arc similar to the one later used in the experiments themselves was the source of light. The thermopile with the open end screened by the red filter was exposed to the light until the galvanometer indicator reached a maximum deflection, which ordinarily took about 30 seconds. The number of divisions through which the spot of light reflected from the galvanometer mirror was displaced on the scale was noted. This was repeated six times, and the average deflection recorded. The other filters were then used in turn to screen the thermopile, and thermopile and screen moved to such a distance from the arc that the displacement of the galvanometer indicator was approximately equal in each case to that produced by the red filter. This distance was also found for the thermopile when screened by a piece of clear glass, which represented the distance of the control from the source. For the experiments the quantity of light used could be varied for the whole set of screens by multiplying or dividing these distances by the same number, and the intensity in all the boxes would remain equal. The actual amount of light in meter-candles can be measured by means of a photometer. Then from the law of inverse squares, namely, that the intensity of light per unit surface varies inversely as the square of the distance from the source, the intensity at any distance from the arc can be computed.

The calibration of the set of screens was repeated seven times, or until satisfactory checks of the distances were obtained. With some thermopiles of less rapid action than the one used here, it is impossible to get results by waiting for the galvanometer indicator to come to a steady state. In such a case the deflections produced by exposure to the light for equal intervals of time can be compared.

A series of measurements for five second exposures agreed very well with those obtained by the other method.

The absolute intensities of the light behind the colored screens were measured with a Sharp-Millar photometer.[1] Since at the prescribed distances all are equal to the white light control, there remained only to measure the intensity of the arc at the distance of the control. For the experiments the distances obtained in the calibration were divided by four, so that the intensity of the arc with the photometer 85 cm. away was measured. It was 2050 meter-candles. This must be corrected for the absorption of the light by the glass of the filters. To get this "absorption coefficient," the intensity of a light was measured both with and without a screen of clear glass equal in thickness to that of the filters. A Lummer-Brodhun photometer was used for this determination. It was found that glass 1.5 mm. thick absorbed 12 per cent of the light falling upon it. To obtain the intensity of the lights as actually transmitted by the light filters, it was necessary therefore to take 88 per cent of the reading of the photometer (2050), which was 1804 meter-candles. Of course the light stimuli acting on organisms in water in the culture dishes were still less, owing to absorption by the glass of the dish and of the water.

TABLE I

DISTANCES AT WHICH INTENSITIES OF LIGHT FROM AN ELECTRIC ARC TRANSMITTED BY WRATTEN LIGHT FILTERS ARE EQUAL

Filter no.	Wave lengths in Ångstrom units*	Color	Distance from light in cm.	Intensity in meter-candles
70.....	6600–7000	Red	320÷4=80.0	1804
71.....	6200–6800	Red	275÷4=68.7	1804
72.....	5900–6200	Orange	230÷4=57.5	1804
73.....	5600–5900	Yellow	250÷4=62.5	1804
74.....	5200–5600	Green	280÷4=70.0	1804
75.....	4700–5200	Blue	250÷4=62.5	1804
76.....	4000–4700	Violet	250÷4=62.5	1804
Control.....	4000–7000+	White	340÷4=85.0	1804

* Professor E. P. LEWIS of the Physics Department of the University of California very kindly made these wave-length determinations.

The lack of agreement between these values and the energy curve of the spectrum is probably due mainly to the peculiar

[1] I am indebted to Mr. W. C. POMEROY of the Physics Department of the University of California for these determinations.

absorption of the filters, but also partly to the fact that they do not all transmit the same number of wave lengths.

Polarity

The power of external factors to determine the polarity of a germinating spore is, without doubt, the power to orient the spindle of the first dividing nucleus, if, as in the case of *Fucus*, that polarity is established by the direction of the first cleavage plane. The work on such orientations is very limited, and has often yielded negative results. DRIETSCH established the polarity of sea-urchin eggs by subjecting them to pressure, the spindle forming parallel to the flattened sides of the egg. A number of investigators have found that unilateral white light will establish the polarity of the spores of some of the lower plants by causing the first cleavage plane to be formed perpendicular to the direction of the incident light. Without exception the cell on the darker side of the spore becomes the rhizoidal cell, the other being apical. Equal illumination on all sides retards or prevents germination. This has been demonstrated in *Equisetum*, *Fucus*, *Ascophyllum*, *Pelvetia*, *Dictyota*, *Laurencia*, *Cystoseira*, *Anthoceros*, *Fimbriaria*, *Gymnogramme*, and *Puccinia*. It has been proved that gravity and contact cannot establish the polarity of these spores.

The first report of this phenomenon of polarity established by light is that of STAHL (**20**), who worked on *Equisetum*. He found that the first wall is formed perpendicular to light rays striking the spores on one side only, and that if all sides are illuminated by rotating the spores on a clinostat, the formation of the wall is retarded or prevented. The cell on the shaded side of the spore becomes the rhizoidal cell. In darkness the formation of the first wall follows no rule, and the rhizoids extend in every direction. STAHL refers to earlier work on *Marsilia* and *Chara* which indicates that gravity is a controlling factor in the orientation of the first division plane.

ROSENVINGE (**19**) showed that in *Fucus spiralis* there is no relation between gravity and the first division plane, nor does contact with a solid body have any effect. He got the same orientation to light in *Ascophyllum* and *Fucus* that STAHL did

with *Equisetum*, but with puzzling exceptions. Where the spores were in groups, the cell toward the interior of the group became the rhizoidal cell; and in the lower part of hanging drops the rhizoids appeared on the upper side of the spore regardless of the light direction. He concluded, therefore, that not only light but a difference in the concentration of oxygen on the two sides of the spores could determine their polarity. He says that as a result of their respiration the water in the center of the groups of spores is less rich in oxygen, with the result that the rhizoids are formed on that side. In support of this theory is the fact that although light can determine the polarity of all the species studied except *Fucus serratus*, namely, *Ascophyllum nodosum*, *Fucus vesciculosus*, *F. spiralis*, and *Pelvetia canaliculata*, their sensitivity to light differs, and the oxygen factor or internal causes produce frequent exceptions in all but *Pelvetia*. The rhizoids of the latter species are always formed on the darker side of the spore, and this is the one species in which the egg is surrounded by an oogonial wall which might prevent any of the effects of varying oxygen concentration that can act more potently than light on the spores of the other species. ROSENVINGE quotes KNY as finding that neither light, gravity, nor contact can influence the point of origin of the pollen tube from pollen grains, but that in the neighborhood of other grains the tube will be sent out from the side away from them, on which side the supply of oxygen or nutritive elements would be greater.

FARMER and WILLIAMS (6) state that if *Fucus* spores are illuminated on all sides they tend to remain spherical instead of producing a rhizoid by the elongation of one of the two cells. Again (7) they experimented with one-sided illumination, with the usual result that most of the rhizoids originated on the shaded side of the spore and the others were turned that way. The fact that some grew out at an angle to the incident light was attributed to "the character of the egg itself."

WINKLER (21) found the same orienting effect of light on the spores of *Cystoseira barbata*, but failed to find any effect of a difference in the oxygen content of the water. He also said that gravity and contact are not factors in the establishment of the polarity of the sporelings. He found that it is determined during

the first four hours of illumination and cannot be changed afterward by any change in the direction of the incident light. He concludes, therefore, that light can orient the spore only during fertilization.

PEIRCE and RANDOLPH (18) performed one-sided illumination experiments with *Dictyota*, *Dictyopteris*, *Laurencia*, and *Cystoseira*, and pointed out the certainty of the action of other factors besides light, because rhizoids are formed in the dark and in all-sided illumination. They said that although WINKLER (21) suggested the possibility of stopping germination by changing the direction of light every three hours, it could not be done with *Dictyopteris*. They emphasized the possibility of influences preceding the illumination affecting the polarity. Later, PEIRCE (17) demonstrated this same phenomenon, that is, the orientation of the first cleavage plane and the establishment of the apical and basal cells by light, with spores of *Anthoceros*, *Fimbriaria*, and *Gymnogramme*.

The work of FROMME (8) on the urediniospores of *Puccinia rhamni* is interesting because it refutes the idea that the orientation of the sporelings by light is due to the power of one-sided illumination to cause an aggregation of chloroplasts. He said that in darkness the germ tube grew from any side of the spore, but that in unilateral light the tubes almost always issued from the darker side of the spore.

In order to obtain an abundance of spores for experimental work, the following procedure suggested by Dr. N. L. GARDNER was followed. The fruiting plants of *Fucus inflatus*[2] were collected at Sausilito at low tide one day and kept overnight in damp newspapers. The next morning they were dried slightly by exposing them to the air for from 15 to 30 minutes. The fruiting tips were then cut off and submerged in sea water in the culture dishes. After about 15 minutes many eggs and sperms settled to the bottom of the dish, or could be scraped off into the water, and the piece of plant was then removed. The fact that *Fucus inflatus* is a monoecious species makes it impossible to tell the exact time of fertilization, but it occurs soon after the eggs escape from the oogonial sac into the water. The sperms at this time can be seen

[2] Although the identification of this species is not certain, it is thought by Dr. N. L. GARDNER to be most probably *Fucus inflatus*.

escaping from the antheridia and swimming rapidly around the eggs, then scattering as, presumably, one of them succeeds in entering. The first cross-wall can be seen very plainly 24 hours after the cultures are started. The mucilage accompanying the eggs causes them to adhere so firmly to the bottom of the dish that it is not necessary to use solid media to keep the sporelings from being displaced when the cultures are moved to the microscope stage for examination.

The original plan for determining which wave lengths are the effective ones in the orienting action of white light upon the germinating spores was to use the Wratten filter screens with the electric arc. With this purpose in view, the set of seven screens borrowed from the physics department were analyzed as to wave-length transmission, and the distances from the arc were found at which the dark boxes with these screens as windows should be placed to make the light intensity equal in all. After repeated failures to get the spores to germinate on account of the high temperatures produced by the naked arc at the distances at which it was necessary to place the cultures, this source of light was abandoned, as was also a 1000-watt Tungsten globe for the same reason. Neither would the spores germinate when the boxes were placed in direct sunlight, as the heating effect was too strong, especially behind the red filters. The first positive results were obtained with a mercury vapor lamp behind the blue, violet, and ultra violet screens, the violet transmitting waves of 4000–4700 Ångstrom units, the blue 4700–5200. The same effect was produced in these lights as is produced by white light, namely, the first cleavage planes formed perpendicular to the direction of the incident light, and the cell on the darker side of the spore became the rhizoidal cell. This effect was not produced behind the green, yellow, and red screens, behind which cultures were exposed at the same time. This experiment was unsatisfactory, inasmuch as it offered no way of proving that the other wave lengths were less capable of producing the phenomenon than were those of the blue end of the spectrum, because the blue light is so much more intense in this lamp than are the red, yellow, and green. The problem was then dropped for several months, during which time no

way was found of obtaining red light sufficiently intense which did not kill the spores by its heating effect.

Finally a set of three Wratten screens was obtained consisting of a red, a green, and a blue filter, which seemed less dense than those used before. The wave lengths to which each screen was permeable were determined by means of a direct-vision spectroscope. The experiment was then repeated, using the light from large north windows facing an open field. Thus on the bright summer days used for the experiments there was a maximum intensity of indirect light available which had no disastrous heating effect. Results were immediate and decisive. The light orientation of the axes of the young sporelings was as striking in the green and blue lights as in the control in white light (fig. 1), but entirely lacking behind the red screen where the germination and development proceeded just as they did in the control in darkness. Table II

TABLE II

WAVE LENGTHS OF LIGHT WHICH CAN ESTABLISH THE POLARITY OF GERMINATING *Fucus* SPORES IN UNILATERAL ILLUMINATION

Color of light	Wave lengths transmitted	Appearance of polarity in sporelings
Blue	4000–5000 6100–6300	+
Green	4800–6000	+
Red	5800–7000	−
White (control)	4000–7000	+

indicates that from the limits of the visible blue to somewhere in the green of the spectrum wave lengths of light can establish the polarity of *Fucus* plants, while from this point in the green to the boundary of the red they cannot (figs. 1, 2). Just where, in the green, light ceases to be effective could not be determined with these screens.

The intensities of these colored lights were not equal, but the red, as in any daylight source, was many times stronger than that of the green or blue, and therefore it is all the more significant that with so much greater energy the long red rays cannot produce the reactions of the shorter blue ones. We can say definitely that

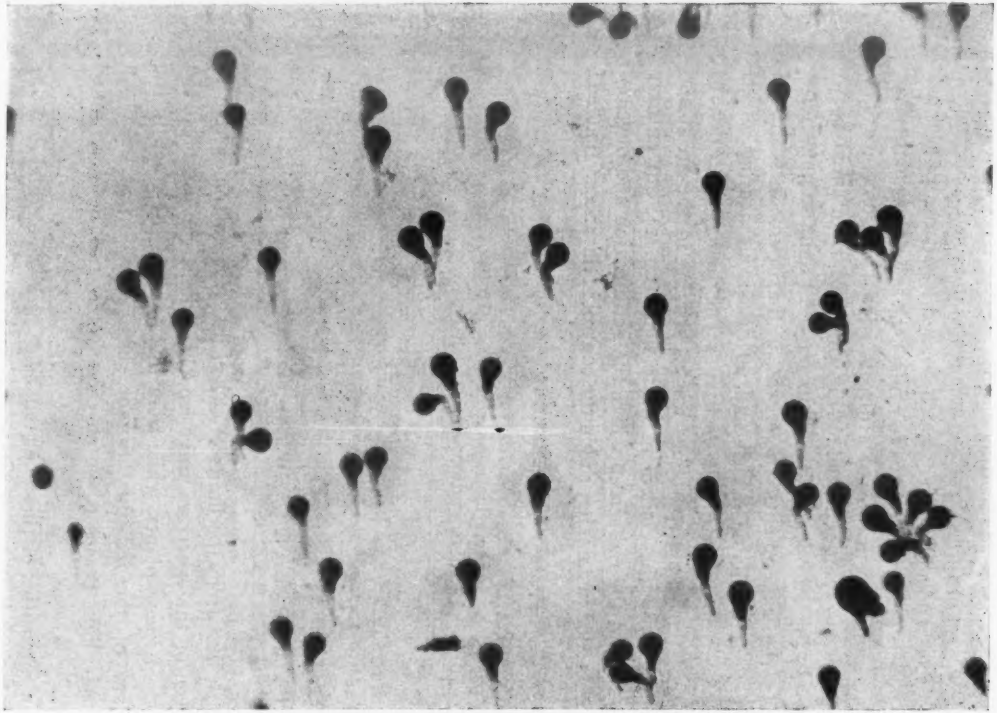

FIG. 1.—Effect of unilateral illumination with white light or its effective components on germinating *Fucus inflatus* spores, rhizoids growing from darker side of spore and extending parallel to direction of incident light; note groups of 2, 3, and 5 spores for which some chemical stimulus originating in activities of adjacent spores proved stronger than light stimulus, producing phenomenon of group orientation; ×50.

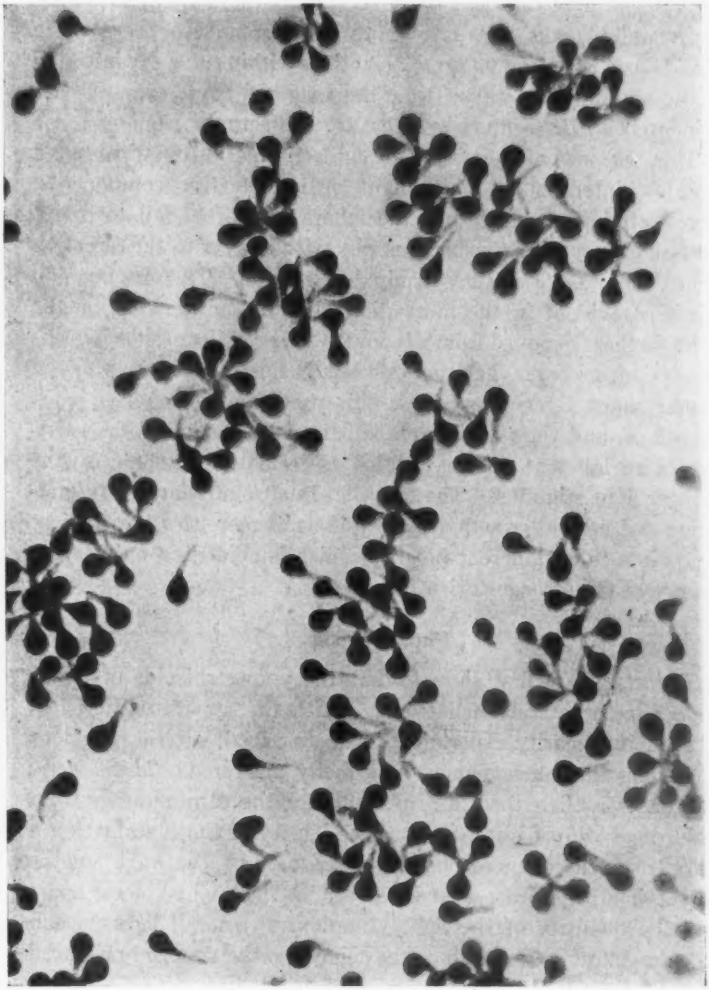

FIG. 2.—Group orientation of *Fucus inflatus* spores germinated in darkness; ×50

the power of light to orient the first cleavage planes of germinating spores and to cause the cell on the darker side to become the rhizoidal cell is dependent on the reactions within the spore initiated by the short actinic rays; that the long red rays, even though their intensity be so much greater, are ineffectual.

This response of the spores to light stimulation requires but a very low intensity of white light or its effective components. Spores left in open dishes in the laboratory rarely fail to orient themselves in a most conspicuous way with respect to the direction of the windows, nor do they require bright light. The phenomenon is just as evident in the more dimly lighted cultures left on the tables farthest removed from windows. Every culture showing this orientation, however, has more or less frequent exceptions to the general rule. Every worker on this problem has reported such exceptions, and they have been explained by assuming the existence of an inherent polarity, which as a rule is overcome by the stronger light stimulus. The fact that in absolute darkness germination and normal growth are as rapid as or more so than in light also points to an inherent polarity which is evidenced only in the absence of the stronger orienting agents.

Phototropism

WINKLER (21) first showed that the young rhizoids of *Fucus inflatus* are negatively heliotropic. With the apparatus designed for the light polarity experiments just described, several questions concerning this phenomenon were easily answered. These were: (1) what wave lengths are responsible for the turning away from a source of white light? (2) is the intensity of the illumination a factor, or is the phenomenon controlled only by the wave lengths acting; in other words, what is the rôle of the quality factor apart from the quantity of the light stimulus? (3) do all lights which have any effect at all produce the same negative tropism produced by white light?

Although the light emitted by the electric arc was too low in intensity after passing through the dense filters to establish the polarity of the young *Fucus* plants, it was noticed very early in the investigation that behind the blue and violet filters this light

was strong enough to cause all the rhizoids to turn sharply away from it. Experiments were started, therefore, to determine whether those behind the filters transmitting the longer wave lengths would not also show this effect, the intensity of all the lights being kept equal. The spores were germinated in small dishes in darkness, and allowed to grow until the rhizoidal cell had divided at least once. Cultures were then placed in the seven dark boxes behind the original set of seven Wratten filters, and these boxes placed at such distances from the naked arc that the intensity of light behind each was 1804 meter-candles (table I). The illumination was continued 6–7 hours. The next day the cultures were examined to see which ones showed the characteristic negative phototropism. It was found in every case that only those illuminated by the blue and violet light had been so affected, those behind the other filters having their rhizoids unbent, continuing in the direction in which they had started, just as did the control in darkness. With all the intensities the same (1804 meter-candles), therefore, wave lengths capable of producing the negative phototropism so commonly seen after white light illumination are those of 4000–5200 Ångstrom units, all the others having no effect.

TABLE III

WAVE LENGTHS WHICH WITH AN INTENSITY OF 1804 METER-CANDLES PRODUCE A NEGATIVE PHOTOTROPISM IN *Fucus* RHIZOIDS

Filter no.	Color	Wave lengths in Ångstrom units	Distance from arc in cm. at which intensities are equal	Appearance of negative phototropism
70.....	Red	6600–7000	80	−
71.....	Red	6200–6800	68	−
72.....	Orange	5900–6200	57	−
73.....	Yellow	5600–5900	62	−
74.....	Green	5200–5600	70	−
75.....	Blue	4700–5200	62	+
76.....	Violet	4000–4700	63	+
Control.....	White	4000–7000	85	+

The same experiment was tried with sunlight as the source of illumination. The young plants were exposed behind the filters all day in a south window. The same results were obtained as

when the arc was used. Then the experiment was repeated with diffused light. The boxes were placed in an east window for 8 hours. Again the rhizoids in the blue and violet light showed the response, but in addition a considerable but much smaller number were affected in the same way behind the green filter. Later the experiment was repeated with the second set of three filters with which results were obtained in the experiments on light polarity. These, as explained before, were less dense than the first set, and were used with the light from a north window. Here the negative phototropism appeared behind the green and blue filters, but not behind the red. Thus the same filters whose light was found to have the power of establishing the polarity of the germinating spores were also the ones which produced the rhizoidal phototropism (table II). As explained previously with reference to these filters, the intensity of the ineffective red was many times greater than the shorter but more powerful wave lengths. Just where between 4800 and 6000 Ångstrom units the rays cease to be effective it is impossible to tell with these filters, but with the first set it was found that the phototropism occasionally occurred behind the green filter with a range of 5200–5600 Å.U. It is probable, therefore, in view of the fact that in these latter experiments the blue and violet rays of 4000 to 5200 Ångstrom units never failed to produce the phenomenon at equal or less intensities, that the tropic power of light gradually decreases from the violet and blue toward the red end of the spectrum, losing its power at ordinary intensities somewhere around 5600 Å.U. It remains to be seen whether the still longer rays can be made to produce the same effects by increasing their intensity.

Only the growing tips of the rhizoids are sensitive to light. This usually results in a sharp angular turn if the direction of illumination is changed through 90° or 180°. As pointed out by LOEB and others, such tropisms are probably due to the difference in the speed of the chemical reactions going on in the two sides of the growing tip. The first protuberance of the germinating spore is not affected by light striking it from the side; and if it is so illuminated during the early stage of elongation of this cell, the first bend occurs at the cross-wall separating it from the next

rhizoidal cell. In other words, the direction of the first protuberance is at right angles to the first cleavage plane, whatever the direction from which it is subsequently illuminated. After the first cross-wall has formed in the rhizoid, however, a change in the direction of illumination results in an angular bend at the tip.

Group orientation

The preceding experiments have demonstrated the power of light to establish the polarity of germinating spores of *Fucus inflatus*. Yet another factor was found to exert an orienting influence on the spindle no less potent than that of light, that is, the proximity and direction of other germinating spores. ROSENVINGE (19) described this most striking and interesting phenomenon in other species of *Fucus* and in *Ascophyllum*. The first cross-wall forms perpendicular to the direction of the adjacent spore or group of spores, or, if the spore be one of a group, perpendicular to the direction of the center. The cell toward the interior of the group, or toward the source of the stimulus in the case of more isolated cells, becomes the rhizoidal cell (fig. 2). For want of a better term I have called this phenomenon *group orientation*. It is best studied in cultures germinated in darkness, and hence free from orienting effects of light.

This phenomenon is as conspicuous in groups of 2, 3, or 4 eggs as in masses of 50 or 100, so long as they are within the distance of each other through which the stimulus is effective. This distance is usually 0.2–0.3 mm., but occasionally spores as much as 0.5 mm. apart have shown the mutual orienting influence. If there are only two spores concerned, the first cleavage planes are often parallel, and the rhizoids, growing from the inner cells, meet tip to tip. In the small groups of five or six the rhizoids, all growing toward the interior, make rather symmetrical starlike designs. In the larger groups or masses of spores the phenomenon is made evident by the fact that no rhizoid is ever found taking a direction away from the group. Although the finding of many groups of eight lying together just as they escaped from the oogonial sac, and beautifully oriented with respect to each other, suggests an inherent polarity established by the relative positions of the eggs in the

oogonium, the phenomenon appears just the same if these groups are stirred up with the point of a needle before being germinated, so that the original positions are entirely changed.

The direct cause of such orientations is probably the same as that responsible for those produced by light. At least the results of the stimulations are identical, and it seems probable that the ultimate factor is the relative rate of the oxidations proceeding along an axis of the spore. It follows that the energy of light might determine this oxidation gradient in unilaterally illuminated cultures, but in those germinated in darkness the oxygen content of the water on the different sides of the spore, being more exhausted on the side next to other growing spores, might disturb the equilibrium and produce the same sort of a gradient. ROSENVINGE advanced the theory that the phenomenon was produced by a difference in the concentration of oxygen or of nutritive substances on the two sides of the spore. He thought the rhizoid forms on the side toward the center of a group or toward another egg, because as a result of the latter's metabolism the water on that side is less rich in the active substance than on the outer side of the spores. WINKLER, however, working with *Cystoseira barbata*, found that a difference in oxygen concentration which he produced artificially had no such effect on the spores.

This phenomenon, group orientation, is found in cultures germinated in light as well as those in darkness, although not so conspicuous in the former, because the light may be the stronger stimulus for many spores which in its absence would be affected by the orienting stimulus from adjacent spores. Yet the fact that it is always found in cultures germinated in unilateral light, although limited to those spores and groups of spores within a very short distance of each other, shows that within this distance the influence of neighboring spores is stronger than that of light, at least of lights with the intensities of those used in these experiments. In other words, no light was powerful enough to overcome for the more closely grouped spores the chemical stimuli originating in themselves. The relative number of spores oriented by light depends therefore on the intensity and wave-length composition of the light source and the distribution of the spores in the culture. In many

cultures only the more isolated spores will be oriented by light, the others all showing strongly the group orientation. The spores show the greatest individual differences in their relative sensitiveness to light and to the group stimulus. Of two spores lying within about 0.3 mm. of each other, one might be entirely oriented by the adjacent spore, while the other, apparently like it, would show only the action of the light stimulus. In many cases two such spores would seem to show a resultant effect of the two stimuli, so that both would be half turned toward each other, with both rhizoidal cells showing a tendency to take a direction away from the light at the same resultant angle (fig. 1).

The substance or condition originating in the activity of adjacent spores which has so powerful an effect in orienting the first cleavage plane and in determining which cell shall become the rhizoidal cell has no power to cause any chemotropism of the rhizoids after they are started. No rhizoid has been found to have its direction modified by the presence of other spores adjacent to it. In the absence of any light stimulus the rhizoids continue in the direction that they take originally from the spore.

Discussion

CHILD'S (**3, 4**) metabolic gradient theory seems to offer the most satisfactory explanation of the power of environmental factors to establish the polarity of germinating spores. He has demonstrated in many marine plants and animals the existence of "axial susceptibility gradients" which he considers due to a decreasing rate in the metabolic processes from the apical to the basal or posterior end. Such a gradient would be established by differences in the rate at which oxidations and other reactions proceed, and by this disturbance of the equilibrium of the physiological mechanism would determine the basal and apical ends of the organism. CHILD'S explanation is as follows:

> Since extended experiment with the lower animals indicates that the degree of susceptibility to cyanides and to many other agents and conditions is in a general way, and within certain limits, a rough measure of metabolic activity or of certain fundamental metabolic processes, probably primarily the oxidations, these axial differences in susceptibility in the algae are regarded as indicating the existence of axial metabolic gradients. . . . . In the final analysis such a gradient is not self-determined by some sort of organization,

but arises as the result of the differential action of factors external to the protoplasm, cell, or cell mass acted upon. If, for example, an undifferentiated cell or cell mass is stimulated at some point by the action of a factor external to it, the resulting increase in metabolic activity is not limited to the region immediately affected, but a wave of change spreads or is transmitted over or through the protoplasm with decreasing energy, intensity, or physiological effectiveness, until, if the mass be large enough, it becomes inappreciable at a greater or less distance from the point of origin.

In the case of determination of the polarity of a spore by the direction of its illumination, it might be said, therefore, that a gradient is established within it by virtue of the fact that the oxidation reactions proceed more rapidly on the side receiving the greater amount of light energy. This side would become the apical end, if CHILD's supposition is correct that the higher rate is toward the apex, or head, and that in the posterior parts, or in the rhizoids of algae, the rate is least. Thus it might be said that the disturbance of equilibrium within the spore due to the reception of unequal amounts of light energy over its surface produces an oxidation or metabolism gradient which establishes the polarity of the young plant. The spindle is oriented in some unknown way, and the less active of the two cells resulting from the first cleavage is the rhizoidal cell.

Why only the rays of the blue end of the spectrum should have this action is not clear. Possibly the cells exercise a selective absorption such as that described by BOVIE (2) as possessed by *Paramoecium* when acted upon by ultra violet light. Then the differences in the effects of monochromatic lights on *Fucus* spores would be due to differences in penetrating power rather than to "any action specific of wave length." Possibly the energy becomes available to the cell for its effect on oxidations through some photosensitive substance which responds only to the actinic rays.

CHILD has recently found a metabolic gradient or oxidation gradient in these germinating spores, and he finds that the region of highest susceptibility, which he takes to be the region of highest oxidation, is at first at the rhizoidal end, suggesting that the original effect of the light is an inhibition of reactions on the exposed side of the spores. A letter written to the author regarding this point contains the following:

As regards *Fucus*, I found the situation very interesting and very similar to that which exists in some of the lower animals. The egg shows a gradient as soon as polarity is determined, but as you suggested, it might be the region of highest susceptibility, which I believe to be the region of highest oxidation rate, is at first at the rhizoidal end. This makes it look as if the effect of light might be a differential inhibition rather than a differential stimulation. After five or six days, however, the susceptibility of the rhizoid decreases, and at the same time a new region of high susceptibility begins to appear at what is to be the apical end of the thallus, and this becomes and remains the most susceptible region of the plant, and from it a gradient of decreasing susceptibility extends basally to the base of the thallus. It looks as if the outgrowth of the rhizoid represented a rather brief period of high rate of oxidative activity, which soon slows down, and then the apical region of high rate arises, just as a bud, previously inhibited, arises or begins to develop when the activity of the growing tip which inhibited it is decreased. Of course these are at present merely suggestions by way of interpretation of the observed facts.

As for group orientation, it and the effect of light may have a common physiological basis. In both phenomena the controlling force may be gradients of increasing oxidation rates, but with different factors responsible. In the case of the light effect it may be the available energy speeding or retarding metabolic reactions where the wave lengths acting are such that they can be absorbed by the active substances of the cell; or in the case of group orientation, it may be available oxygen or other nutritive substance varying in amount on two sides of the spore as the result of the metabolic processes of adjacent spores.

We must conclude, however, that the attempts at partial explanation of these experiments and observations are far from satisfactory. The application of the oxidation gradient theory can only account for the later aspects of the polarity phenomena, the determination of the apical and basal ends of the germinating spore. The orientation of the first cleavage plane determined by orientation of the spindle of the dividing nucleus is visible evidence of forces existing within the cell, and the control of those forces by light energy and by chemical stimulation in a manner of which there is no hint, and the mechanics of which must remain obscure for the present.

Summary

1. A convenient method for obtaining monochromatic lights of equal intensity is the use of the thermopile and galvanometer

to obtain the relative intensity of the light transmitted by accurate color screens, and the adjustment of the distances of these screens from the light source such that the deflections of the indicator on the galvanometer scale are equal for each exposure of the thermopile screened by the light filters in turn.

2. The effective wave lengths in the establishment of the polarity of *Fucus* spores, the result of whose use for unilateral illumination is the same as that produced by white light (the orientation of the first cleavage plane perpendicular to the direction of the incident light with the cell on the darker side of the spore becoming the rhizoidal cell) are, with the intensity of strong diffused daylight, the shorter rays of the blue end of the spectrum of approximately 4000–5600 Ångstrom units. There is some evidence that ultra violet light can produce the same effect.

3. The negative phototropism of the rhizoids in monochromatic light is also primarily a function of the quality of the light, since, with equal intensity of illumination, the wave lengths of the red end of the spectrum are without effect, while those of 4000–5200 Ångstrom units produce the same phototropism produced by white light.

4. The term "group orientation" is suggested for the phenomenon of the orientation of the first cleavage plane of a dividing spore with reference to the position of adjacent spores, such that it is perpendicular to the direction of the center of a group or of a single spore within the effective radius, with the subsequent development of the cell on the side toward the source of stimulus as the rhizoidal cell.

5. This group orientation reported in other species is a conspicuous phenomenon in every culture of *Fucus inflatus*, the stimulus acting in such orientations being so strong that when spores are separated by as much as 0.2 mm. and often more, light stimuli as a rule fail to overcome it.

6. The chemical stimulus which orients the direction of the first cleavage plane and determines which cell shall become the rhizoidal cell in group orientations has no power to cause a chemotropism of the rhizoids.

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CROWN-GALL OF ALFALFA

O. T. WILSON

(WITH PLATES VII–X)

Introduction

The crown-gall of alfalfa has been known in the United States for only a few years, the first published record of its occurrence in this country being in 1909. It was at that time observed in California by SMITH (**32**). Since then it has been reported from Arizona (**23**), Oregon (**26**), and Utah (**27**). It is probable that it also occurs in other western states. The disease has been known somewhat longer in other countries. PATOUILLARD and VON LAGERHEIM (**28**) in 1895 published the earliest record of its occurrence in connection with an outbreak in Ecuador. In 1902 MAGNUS (**21**) described the general features of the disease and recorded its presence in Germany. It has also been reported from England (**29**), and Italy, Sweden, and Switzerland (**10**).

The lack of detailed information makes it difficult to estimate the economic importance of the disease. Old and powerful stands were killed in the fields of Ecuador (**19**). MAGNUS considered the disease serious at Colmar in Alsace. SALMON (**29**) advised strict precautions against its spread in England. In the United States MCCALLUM (**23**) reported the disease as serious but not widely distributed in Arizona at the time of his observations. SMITH noted it in only a few counties of California. According to O'GARA (**26**), many plants two to seven years of age were destroyed by the parasite in Oregon. In 1915 resolutions were adopted by the American Phytopathological Society (**31**) recognizing the serious possibilities of the disease and recommending its investigation by the government.

It is the purpose of this study to contribute to our knowledge of the life history of the causal organism.

Material and methods

Infected plants were secured from the vicinity of Medford, Oregon. In the winter of 1913–1914 P. J. O'GARA sent specimens,

and in the fall of the year 1914 material was received from Dr. M. P. HENDERSON. Other material was secured from alfalfa plants grown and infected in the greenhouse.

Standard histological and cytological methods were employed for the examination of the material; reference to these will be made in connection with various phases of the observations.

Observations

In 1902 MAGNUS (**21**) described the galls as branching tuberculate structures on the larger secondary roots of *Medicago sativa*. Upon examining cross-sections of the galls, he found large brown regions of irregular form, which proved to be cavities filled with resting spores of the parasite. "Thick-walled strongly encysted mycelium" was found in many of the cavities, but he did not find that the resting spores were attached to the hyphae. The amount of mycelium present in the different cavities varied; it was often entirely lacking. The hyphae were described as continuous or branched, and the protoplasm of the host cells was often completely displaced by these "wandering hyphae." MAGNUS thought that this mycelium might "awake to new life" after the winter resting period. He described the resting spores as spherical with one side flattened; he noted a colorless hyaline cell attached to the flattened side by means of a hyaline process. Many pores were found in the centers of the flattened walls of the spores. No other stages in the life of the parasite were mentioned by MAGNUS, and not all those described were figured. So far as the writer has been able to learn, no subsequent work has been published touching upon the life history of this organism, which has been classified as *Urophlyctis alfalfae* (von Lagerheim) Magnus. Figures of the galls have been published by various writers (**13, 22**), and all agree with MAGNUS' original description.

The alfalfa plants which furnished the material for these observations were several years old. Numerous galls corresponding to the structures described by MAGNUS and others as the crown-gall of alfalfa were found upon the plants (fig. 1). Free-hand sections of such galls upon microscopic examination revealed numerous brownish resting spores, like those figured by MAGNUS,

occupying irregular cavities in the tissues of the gall (fig. 8). If the spores are not scattered in sectioning they completely fill the cavities. They average 40 μ in diameter. When shaken from the cavities they appear as a glistening brown powder.

With rare exceptions the spores are spherical in form, except for the depression on one side (fig. 8). Great irregularities of form may occur, especially near the borders of masses of spores, where the host tissues apparently interfere with the natural conformation of the spores. These irregular spores may conform to the outlines of the host cell occupied (fig. 11). A group of slitlike pores in the depressed surface is normally a prominent feature of the spores (fig. 9). BALLY (**3**) observed similar pores in the walls of the spores of *Urophlyctis Rubsaameni*. The walls of the spores consist of two layers, the outer much heavier than the inner (fig. 6). The outer layer is yellow-brown, glassy in appearance, and brittle, as shown in sectioning. It is very resistant to stains, this quality being characteristic of resting spores of the Chytridiaceae. A positive reaction with phloroglucin indicates that it is lignified, but it does not stain with safranin. The inner layer of the wall is thin and hyaline in appearance; it responds to the zinc chloriodide test for cellulose. In this respect it is like the wall of the sporange of *Olpidium Viciae* described by KUSANO (**17**). It lacks the rigidity and brittleness of the outer layer. Ridges in the inner layer are of frequent occurrence (fig. 13).

In the resting condition the protoplasm of the unstained spore appears to be granular in nature and somewhat vacuolate (fig. 10). The nuclei cannot be distinguished without staining. Flemming's triple stain or Heidenhain's iron-alum-haematoxylin may be employed to bring out the nuclei. Many small nuclei are found to be scattered quite regularly through the cytoplasm of the spores (fig. 15). It is difficult to detect the structure of the nuclei at this stage; it becomes easier as the spore develops into a sporange. There is considerable variation in the response of the nuclei to stains. This is probably related to the difficulty in obtaining very thin sections through the spores. Nuclei which have been sectioned show that the chromatin is usually at the periphery, often concentrated at one side. In some preparations

a nucleole appears clearly (fig. 24). The condition of the resting spore at this stage corresponds to that found by LOEWENTHAL (**20**) in the sporange of *Olpidium Dicksonii* at the very beginning of zoospore formation, when the presence of very numerous tiny nuclei was noted. Before staining, the content of the resting spore appears hyaline and refractive in section. Only the nuclei stain deeply; about each nucleus is a clear region, the clear portions being separated by lightly stained cytoplasm.

It is only in the resting stage that the heavy-walled bodies should be called spores. From the nature of their further development they are clearly potential sporanges. The resting spores of the Chytridiaceae have frequently been called sporanges.

The development of the sporanges was first studied in Van Tieghem cells, distilled water being the medium employed. The spores undergo an immediate change when placed in water. The granular appearance gives way to one in which the cell seems to be filled with small globules of oil. This indicates the first changes leading to the formation of zoospores. If the spores are crushed at this stage, numerous globules of fat are freed which stain with Sudan III or with osmic acid. As development continues, the fat globules in the sporanges apparently become larger and less numerous (fig. 14). This condition continues until the motile zoospores are set free.

In water the wall of the sporange also undergoes an almost immediate change; a swelling of the wall on that side takes place, presumably because of the entrance of water through the pores in the depressed surface. BÜSGEN (**5**) observed a somewhat similar swelling of the wall in the sporanges of *Cladochytrium Butomi*. The sporanges become almost or quite spherical in outline. The pores become cracks, and after a time the outer layer may rupture as the inner content becomes more turgid (fig. 18). Various appearances are brought about by the protrusion of the inner layer of the wall and its contents. Sometimes the outer layer is thrown off like a cap, as BÜSGEN observed in his study of *Cladochytrium*. In the majority of cases, however, the outer layer remains intact. The whole process indicates a softening or gelatinization of the wall as compared with its previous brittle condition.

Just before the exit of the zoospores a motion of the sporange contents is visible. The fatty globules are jostled about although they do not flow together. In a spore which is not viable the fat is frequently congregated into one or two large drops, but such a spore was never seen to develop into a sporange. The motion within the sporange is followed by the rupture or dissolution of the inner wall layer, allowing the zoospores to escape through the openings in the outer layer. The difficulty of observing their direct exit is enhanced by the fact that the porous side of the sporange wall is almost always downward in the hanging drop. The zoospores escape sometimes in groups, but usually singly. Often they seem to have difficulty in locating the pores, and they may swim about in the sporange for a considerable time or even disintegrate there (fig. 19). ATKINSON (1) has observed an interesting amoeboid movement of zoospores within the sporanges of *Rhizophidium globosum* just previous to their escape. Although no such amoeboid movements were seen at this stage in my material, the alternation of resting and active periods is a common occurrence, just as was observed by ATKINSON.

In stained sections the development of the sporanges may be traced in a fairly complete series. Even in the very early stages each nucleus seems to be related to a definite portion of the cytoplasm (fig. 26). The nuclei have never been observed to occur in groups, but are rather uniformly distributed. As development continues, the nuclei decrease in number, as shown by the number present in a cross-section of a sporange. Apparently there is a disintegration of many of the small nuclei, while those which are to take part in the formation of zoospores increase in size. The structure of the latter nuclei also becomes much more clearly differentiated, and they stain with more uniformity (figs. 20, 27). The chromatin is now aggregated in knots connected by slender threads (fig. 16). In most of the preparations the connecting threads are not easily seen; in such cases one or more knots are apparent just within the nuclear membrane (figs. 20, 27); these large nuclei are the centers of zoospore formation. BARRETT (4) found in *Olpidiopsis* nuclei similar to those here described, which served as centers of zoospore formation. The transition stages

from the condition shown in fig. 15 to that in which the spores are delimited have not been clearly followed. Zoospore formation is evidently a very rapid process. With the possible exception of the cilia, the zoospores are quite fully developed before their escape from the sporange. The vacuole and nucleus are quite well defined (figs. 23, 25). LOEWENTHAL (**20**) found a vacuole and well defined nucleus in the zoospore of *Olpidium Dicksonii* before its escape from the sporange. BARRETT observed the vacuole in the zoospore of *Olpidiopsis*, but apparently did not succeed in staining the nucleus. A cleavage of the sporange cytoplasm previous to zoospore formation is apparent in well stained sections. This cleavage begins at the margin of the sporange, and works toward the central region (fig. 22). The process apparently corresponds to that described by HARPER (**16**) for *Woroninella*.

An interesting variation from this development is of quite common occurrence, but has been observed only in fixed and stained material, probably because it is masked in fresh material by peripheral fat globules in the sporange. Soon after the nuclei begin to enlarge in the stages preceding zoospore formation, one of them (sometimes several) undergoes an especially rapid development (figs. 17, 21), becoming separated from the other nuclei by a surrounding portion of denser cytoplasm. This is often quite clearly shown, even in unstained sections. A spore may be clearly delimited about this nucleus, while the other spores are yet in an early stage of development. This spore is much larger than the others formed in the same sporange. All the zoospores are alike in structure, the only marked variation being in size. The formation of several of the large spores in the same sporange is exceptional (fig. 29).

When freed in water, the zoospores exhibit a great variety of movement. Often the long cilium, which is quite clearly visible, seems to impede the movement of the zoospore, which exhibits a violent jerking motion. Irregular gyrations are common. Frequently a zoospore moves back to the sporange from which it escaped and seems to seek an entrance. At times the zoospores move very rapidly from the field of observation in a direct line. The movements become less vigorous after a time; the ellipsoid

or ovoid changes to a spherical form, and the long cilium becomes more plainly visible than before, dragging behind passively. This last described feature has been reported for the motile spores of various Chytridiaceae. Periods of activity alternate with periods of passivity, during which the vacuolated condition is very evident. The fat drop is visible only during the active periods. BARRETT (4) and BUTLER (6) have observed pulsations of the vacuoles in the spores of *Olpidiopsis* at this stage.

The fat drop is the most prominent internal feature of the active zoospore. It may be seen to shift rapidly when the zoospore is in motion. After a period varying from a few minutes to several hours, terminated by sluggish amoeboid movements, the zoospore comes to a final rest and soon disintegrates. It seems likely that the refractive body so commonly observed in the zoospores of the Chytridiaceae is a drop of fat occupying a vacuole. ATKINSON (2) observed the presence of a prominent fat drop in the zoospore of *Rhizophidium brevipes*.

Amoeboid movements of zoospores have frequently been mentioned by investigators of the Chytridiaceae. SCHENK (30) observed this phenomenon as early as 1858. DANGEARD (8) noted that the zoospores of *Chytridium xylophilum* creep like amoebae. BÜSGEN (5) observed similar movements in his study of *Cladochytrium Butomi*.

I did not directly observe the exit of the large zoospores from the sporanges, but they were seen in considerable numbers moving slowly about in the water, remaining close to the sporanges from which they had emerged. The content of these large zoospores appears to be more granular than that of the smaller ones, which latter appear hyaline in water. Very soon after their exit the large zoospores are surrounded by the smaller ones. One or several of the latter move swiftly toward a large zoospore and become attached to it. As many as five small zoospores have been seen adhering to one large spore, but in all cases only one remains attached. There seems to be no uniformity as regards the point of attachment. The small zoospore which remains attached loses its cilium just at the time of contact. KUSANO (17) reported a resorption of the cilium at this stage in *Olpidium*. After a small

zoospore has become permanently attached to a large one, the latter continues to move about for a time before coming to rest. An amoeboid form is finally assumed, and disintegration soon follows.

Fusion of zoospores has been reported but rarely in the Chytridiaceae. Probably this is because of the very limited observations upon the motile stages. FISCH (14) observed and figured a fusion of zoospores for *Reesia amoeboides*. DANGEARD (9) noted an apparent fusion in *Sphaerita endogena*. KUSANO observed a clear case of fusion in *Olpidium*, the fusing cells being similar in size. The observations of SOROKIN (33) upon *Tetrachytrium*, of ATKINSON (2) upon *Lagenidium*, and of VON LAGERHEIM (18) upon *Rhodochytrium* should also be mentioned in this connection.

The motile spores were fixed and stained upon the cover slip by the use of osmic acid and gentian violet. Each spore was found to have a short cilium as well as the long one visible in the water (fig. 28). The motion of the zoospore, after the long cilium becomes passive, is probably due to the activity of the shorter cilium. It seems possible that the uniciliate condition is not so common among the Chytridiaceae as has been thought. Care in staining and observation is necessary for a successful determination of the number of cilia. CORNU (7) found only one cilium borne by the motile spore of *Olpidiopsis*. FISCHER (15) later found two, which observation was confirmed by BARRETT (4). Cilia of unequal length on the same zoospore have been reported and illustrated for various members of the family, as for example *Sphaerita endogena* (9).

The cilia of the motile cells of the organism under consideration are attached at the same end of the zoospore to what seems to be a platelike thickening of the membrane. The manner of attachment is much more clearly seen in the larger zoospores (figs. 31, 32). The oil vacuole is near the place of attachment of the cilia (fig. 40). The nucleus lies back of the vacuole, imbedded in the cytoplasm. From the cilia to the nucleus there is a connecting, cone-shaped, apparently fibrous structure, which extends through the vacuole (figs. 31, 32). Fig. 25 shows a general similarity to the zoospore structure described by LOEWENTHAL (20) for *Olpidium*, and figured by NOWAKOWSKI (25) for *Polyphagus Euglenae*.

Fusion stages may be quite clearly followed in material fixed and stained on the slide. Fig. 28 shows two zoospores, a large one and a small one, apparently in the same condition as when freed from the sporange (see fig. 30). The nuclei of stained zoospores often appear to be within a vacuole because of their position when fixed to the slide. The larger zoospore, either before or at the approach of the smaller one, may put out one or more projections in the direction of the latter (fig. 33). Fusion may take place at the apex of such a projection (figs. 34, 36). The two nuclei may be seen within the larger body following the fusion (fig. 35). The fusion of the nuclei has not been observed. Figs. 37 and 38 possibly indicate stages following a fusion succeeded by a division of the fusion nucleus. Many small nuclei appear in the body of the organism at this stage (fig. 37), and there is some evidence of a cell multiplication by budding (figs. 38, 44, 51). It is entirely possible, however, that a multiplication of the nuclei in either the large or the small motile bodies may occur without any nuclear fusion. The observations at this point do not justify very definite conclusions, since the staining of the zoospores *in toto* makes the following of the nuclear phenomena decidedly difficult. There seem, however, to be some grounds for maintaining that the phenomena just described constitute a true case of heterogamy. One cell is characterized by a large body, slow movements, and a nucleus of average proportional size. On the other hand, the smaller cells are swift of movement and have nuclei very large in proportion to their size. The oil drop probably serves as reserve food for the temporary nutrition of either gamete in case fusion does not occur, or of the zygote in case it does occur.

In order to secure the development of the zoospores upon the host, young alfalfa seedlings were carefully washed and introduced into small vessels containing sterile distilled water, so that only the roots and the lower parts of the stem were immersed. A gall from an infected plant was carefully washed, crushed, and introduced into the water. After one day the seedlings were removed from the water, crushed under cover slips on slides, and examined for evidence of the presence of the parasite. On and in the tissues of the host which had been at the water level were numerous

amoeboid bodies, as well as zoospores that still retained their characteristic form. The cilia, however, were no longer to be seen. The amoeboid bodies were watched for some hours and were seen to become clustered and to move in masses; they could also be seen to bud at times, much as in the case of the ciliated cell shown in fig. 38. Budding of the amoebae in the infection stage has been reported to occur in *Plasmodiophora* (11). Seedlings left under these conditions developed small galls at the bases of the secondary roots.

To secure stained preparations of the plasmodium in its early stages, young alfalfa seedlings were grown in a pot in which a badly infected mature plant was also growing. At various times seedlings were removed and examined. Small galls were soon found at the bases of the secondary roots of some of the seedlings. As soon as the plants began to produce crown buds, galls appeared upon the crown (fig. 3). These young galls were fixed, imbedded, sectioned, and examined after various staining processes. The plasmodium of the parasite was found to be widely extended in the galls. An amoeboid or plasmodial vegetative condition of the parasite within the host has often been noted in members of the Chytridiaceae. FISCHER (15) and BARRETT (4) have observed it in species of *Olpidiopsis*. CORNU (7) observed it, as well as the amoeboid movement of the zoospores, in various members of the family. He also noted a suggestion of cleavage in what he called the plasmodium of *Rozella*, and suggested its formation by the union of many amoeboid zoospores. FISCHER went so far as to classify several genera upon the basis of differences in the plasmodia. He observed that the protoplasm of the parasite mingled intimately with that of the host and gradually displaced it. FISCH'S (14) description of *Reesia amoeboides* is a striking suggestion of the near relationship of the group to the Myxomycetes. A secondary infection of cells occupied by the plasmodium is of common occurrence. As a result, the cells of the galls are often occupied, not only by the plasmodium, but also by the earlier stages of the parasite (figs. 39, 41).

The nuclei in the plasmodium are very numerous, corresponding quite closely in size and appearance to those in the resting spores

(figs. 42, 43, 45). The central clear portion surrounded by peripheral chromatin is characteristic of their appearance, although in many cases the whole nucleus takes up the chromatin stains. The cytoplasm of the plasmodium is slightly granular and stains very lightly. It has not yet been possible to keep the plants infected in the greenhouse long enough to secure the development of the resting spores within the galls.

In order to examine the plasmodia in older galls, sections were stained with the triple stain and with haematoxylin. Only comparatively young galls were sectioned, as considerable foreign mycelium was found to be present in the older galls. Infection by other fungi, and the consequent presence of a foreign mycelium which has no connection with the organism causing the galls, is to be expected because of the contact of the infected parts with the soil. Sections of smooth intact galls showed no such signs of foreign contamination. The same type of plasmodium, however, that was found on the exterior of seedlings exposed to infection, and within the tiny galls formed upon seedlings grown in infected soil, was also found throughout the cells of the galls containing resting spores. In marginal cells of some galls the amoeboid cells were found in numbers (fig 47), preceding the formation of a plasmodium such as is shown in fig. 5.

In some cases the plasmodium forms an irregular streaming mass, forcing its way through and between the cells of the gall tissue (fig. 45). In other cases it ramifies through the cells as a network of naked protoplasm (fig. 42). Multiplication of the plasmodia may occur in these stages by budding and fragmentation (fig. 51). The walls of the host cells are often dissolved or gelatinized in advance of the main body of a plasmodial mass, presumably by enzymatic action (fig. 4).

The resting spores are formed in cavities or pockets occupied by the parasite. Remains of host walls are mingled with the plasmodial masses of the parasite, and a clear staining of the material at this stage is almost unattainable. A yellow coloration pervades the unstained content of the pockets previous to the formation of the spores. At the time of spore formation the yellow coloration is limited to the outer walls of the spores, their content being

clear and refractive. Just how the formation of the spores takes place has not been determined.

Around the margins of the larger cavities containing spores the naked protoplasm of the plasmodium may be found in a more or less shrunken condition. The similarity of this protoplasm to that within the spores is convincing evidence of the origin of the spores from the plasmodium, the most noticeable difference being in the more regular arrangement of the nuclei within the spores. Isolated host cells or groups of cells may be occupied individually by separate plasmodia; it would seem that these small plasmodia may develop walls about themselves and become resting spores. The subject of spore formation is in need of very careful and prolonged investigation.

Primary infection, the actual entrance of the parasite into an uninfected host, has not yet been observed. The fact that the parts invaded are the adventitious buds and the secondary roots in the very earliest stages of development minimizes the chances of such an observation. There is little doubt, however, that either the zoospores before fusion, the zygotes, or the plasmodia formed on the surface of the host may penetrate the embryonic host tissues. In young bud galls cases have been found in which practically every cell of the growing tip was occupied by the parasite (fig. 7). From this figure it will be seen that the infecting cells of the parasite vary greatly in form and size after penetration. Often they are found in contact with the host nucleus, the latter being still intact. Sometimes a host cell contains several of the invading cells. Sometimes a fusion of a number of these invading cells, preparatory to the formation of a larger plasmodium and the breaking down of the separating host cell walls, may be noted. All these features are illustrated in fig. 7.

The discussion of the studies upon the life history of the parasitic organism may be concluded by a brief reference to the subject of nuclear divisions in the different stages. Had division figures not been found, identification of the nuclei in the plasmodium would have been questionable, since these nuclei are very small and details of their structure are not easily distinguished. Division figures, however, are very common in some of the prepara-

tions, and are easily recognized as such upon careful examination. The process of division is clearly mitotic (figs. 46, 48–50, 53–57). Dark bodies suggesting centrosomes are commonly visible in the metaphases at the poles of the spindles (figs. 46, 50). The early divisions of the nuclei in the amoeboid infection stages have been followed with only partial success. The figures correspond to those seen in the later divisions, but average considerably larger (fig. 44). The centrosomes, if the dark bodies at the poles of the spindles are to be called such, are a constant feature in good preparations. Clearly recognizable division figures within the sporange have been found less frequently. There is some evidence that a division occurs while the nuclei are yet very small (fig. 15), and much better evidence of a division just preceding the formation of zoospores (figs. 17, 48). Whether these are in the nature of reduction divisions must be left for future determination. Careful study has failed to lead to a definite conclusion as to the chromosome number. In the divisions immediately preceding the formation of the zoospores the number of chromatic bodies seen is apparently four (fig. 48). In all the division figures observed, at whatever stage, the spindle seems to be intranuclear, corresponding to the findings of other investigators of members of the Chytridiaceae.

Classification of parasite

VON LAGERHEIM (**19, 28**), who first noted the occurrence of the crown-gall of alfalfa, classed it in the genus *Cladochytrium* with the specific name *alfalfae*. MAGNUS (**21**), in his article of 1902, gave strong reasons for removing the organism from that genus and referred it to the genus *Urophlyctis*, retaining the specific name given by VON LAGERHEIM. The terminology *Urophlyctis alfalfae* (von Lagerheim) Magnus has been generally accepted in later works. Although MAGNUS was right in removing the parasite from the genus *Cladochytrium*, it is doubtful whether he was justified in placing it in the genus *Urophlyctis*, on the basis of his limited observations. The description of the genus *Urophlyctis* as given in Saccardo's Sylloge Fungorum (**7**: p. 303) is as follows:

Urophlyctis Schroet. Krypt. Fl. Schles. Pilze. p. 197. (Etym. *oura* cauda et *phlyctis* bulla.) Zoosporangia sessilia in plantis vivis, fasciculis filamentorum

immersa. Sporangia perdurantia intra cellulas plantarum viventium e mycelio filiformi perforatas formata, copulatione cellularum similium orta. Cellulae alterius protoplasma in alteram effunditur, haec vero crescit et membrana crassa cincta in sporangium perdurans mutatur.

The studies of the alfalfa crown-gall organism by MAGNUS did not establish the characters cited by SACCARDO. The descriptions of the genus as given by ENGLER and PRANTL (12) and by MIGULA (24) also include characters that MAGNUS apparently did not verify.

The writer has failed to observe, in the organism studied, the characters ascribed to the genus *Urophlyctis*. In view of the incompleteness of the results, no change of classification is suggested at the present time. It seems highly desirable, however, that careful investigations be made of the various organisms referred to this genus. Possibly it may prove necessary to discard some of the previously used diagnostic characters and to redescribe the genus on the basis of fuller observations. The relationships of the Chytridiaceae are in an unsettled state. There have been many suggestions in the literature that would lead one to question whether or not this family finds its proper place among the Phycomycetes. The studies upon which classification has been based in many cases have been very superficial, and few efforts have been made to follow out complete life histories. Cases in which an amoeboid or plasmodial stage has been noted, with the absence of anything resembling mycelium excepting naked threads of protoplasm, furnish reason to suspect that the family is more closely related to the Myxomycetes than to the Phycomycetes.

Summary

1. The resting spores, placed in water cultures, develop into sporanges.

2. Within these sporanges are formed motile zoospores of two sizes; frequently one large zoospore and many small ones are formed in the same sporange.

3. One or several small zoospores may become attached to one large zoospore. Only one remains permanently attached. There is some evidence that this attachment is related to a sexual fusion.

4. The movement of the large zoospore continues after the attachment of the small one.

5. The small zoospores, the large zoospores, and the united zoospores (zygotes?) become amoeboid after a period of motility.

6. In the amoeboid state, singly or in groups, these bodies may be observed upon the surface of the host.

7. In infected soil young alfalfa seedlings develop galls in which plasmodia are found.

8. In older galls similar plasmodia are found, which ramify through the tissues of the gall.

9. The resting spores are formed in cavities within the tissues of the galls.

10. The cytoplasmic and nuclear contents of the resting spores in the dormant condition correspond to those of the plasmodium in the stage immediately preceding the formation of resting spores.

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EXPLANATION OF PLATES VII–X

With the exception of plate VII, the figures were drawn with the aid of an Abbé camera lucida at table level. Leitz oculars and objectives were used, giving the magnifications indicated.

PLATE VII

FIG. 1.—Alfalfa plant badly infected with crown-gall; ×½.

FIG. 2.—Single galls; ×1.

FIG. 3.—Seedling infected in greenhouse, galls beginning to develop at crown; ×1.

FIG. 4.—Section showing action of parasite upon walls of host cells in advanced stage; parenchyma tissue; ×300.

FIG. 5.—Plasmodium on border of gall; ×300.

FIG. 6.—Sections of resting spores showing outer and inner layers of wall; ×130.

FIG. 7.—Section of young gall on seedling showing cells of parasite in host cells; some of former fused into tiny plasmodia, others in contact with host nuclei; ×300.

FIG. 8.—Section of gall showing cavities and resting spores of parasite.

PLATE VIII

FIG. 9.—Unstained resting spore, view of hollowed surface; ×750.

FIG. 10.—Unstained resting spore, side view; ×750.

FIG. 11.—Resting spore conforming to host cell in which it has developed; ×750.

FIG. 12.—Unstained resting spore showing marginal vacuoles; ×750.

FIG. 13.—Irregular relation of walls of resting spore; ×1800.

FIG. 14.—Unstained sporange when first put into hanging-drop culture; ×750.

FIG. 15.—Section of resting spore; may be stage just following resting period; ×1800.

FIG. 16.—Group of nuclei in sporange; chromatin in marginal aggregations; ×1800.

FIG. 17.—Division figures in sporange, apparently just preceding zoospore formation; large nucleus destined to be nucleus of large zoospore; ×1800.

FIG. 18.—Spore formation about completed, unstained; note rupture of outer wall; ×750.

FIG. 19.—Unstained sporange containing a few zoospores; most of those formed already escaped through prominent opening; ×750.

FIG. 20.—Section of sporange just preceding formation of zoospores; ×1800.

FIG. 21.—Section of sporange showing large nucleus which will be included in large zoospore; ×1800.

FIG. 22.—Marginal portion of sporange showing beginning of cleavage into zoospores; ×1800.

FIG. 23.—Zoospores almost fully formed in sporange; ×1800.

FIG. 24.—Nuclei of sporange showing nucleoles; ×1800.

FIG. 25.—Zoospore just before exit from sporange; ×1800.

FIG. 26.—Nuclei of resting condition of spore.

FIG. 27.—Section of sporange dividing into zoospores; note characteristic arrangement of chromatin in knot at one end or side of nucleus; ×1800.

PLATE IX

FIG. 28.—Free zoospores of two sizes; ×1800.

FIG. 29.—Group of large zoospores formed in same sporange; ×1800.

FIG. 30.—Large and small zoospore within same sporange; ×1800.

FIGS. 31, 32.—Two large zoospores; ×1800.

FIG. 33.—Large and small zoospore showing projection of former toward latter; position may be accidental; ×1800.

FIGS. 34, 36.—Attachment of small zoospores to large; ×1800.

FIG. 35.—Binucleate zygospore following fusion(?); ×1800.

FIG. 37.—Amoeboidal stage following fusion, or perhaps developing without fusion; nuclei have multiplied; ×1800.

FIG. 38.—Apparent budding in amoeboid stage; ×1800.

FIGS. 39, 41.—Plasmodium and young infecting amoebulae in same host cells; ×1800.

FIG. 40.—Free zoospore, small size; ×1800.

PLATE X

FIG. 42.—Plasmodium spreading through tissue of host; host nucleus visible; ×1800.

FIG. 43.—Plasmodium breaking through wall of host cell; ×1800.

FIG. 44.—Amoebulae within tissues of host; note nuclei in division; ×1800.

FIG. 45.—Note as for fig. 42; no host nucleus visible.

FIG. 46.—Nuclei of plasmodium in division; ×1800.

FIG. 47.—Amoebulae massed in marginal cells of gall; ×1800.

FIG. 48.—Division figures in sporange; ×1800.

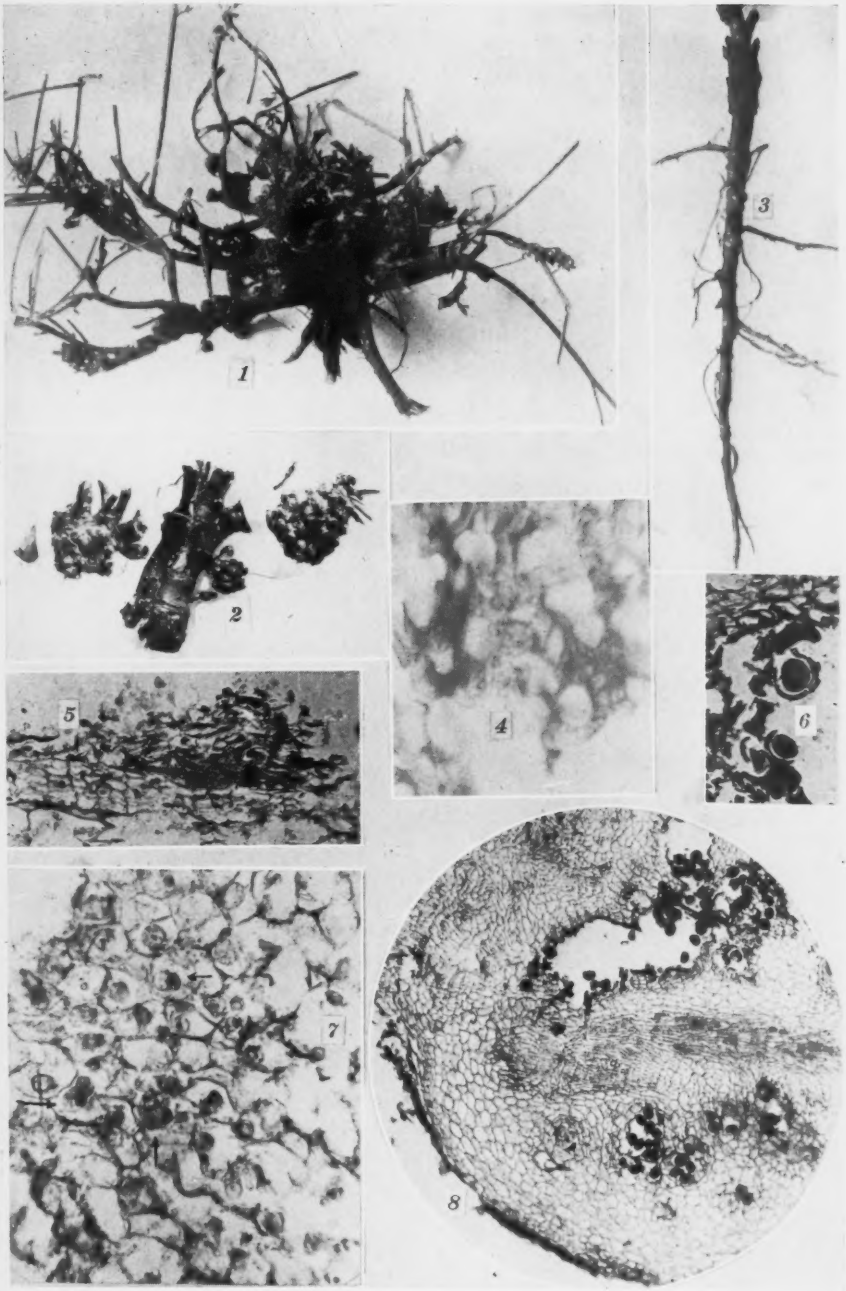
FIG. 49.—Prochromosomes(?) in nuclei of sporange; ×1800.

FIGS. 50, 53.—Anaphases in plasmodium; ×1800.

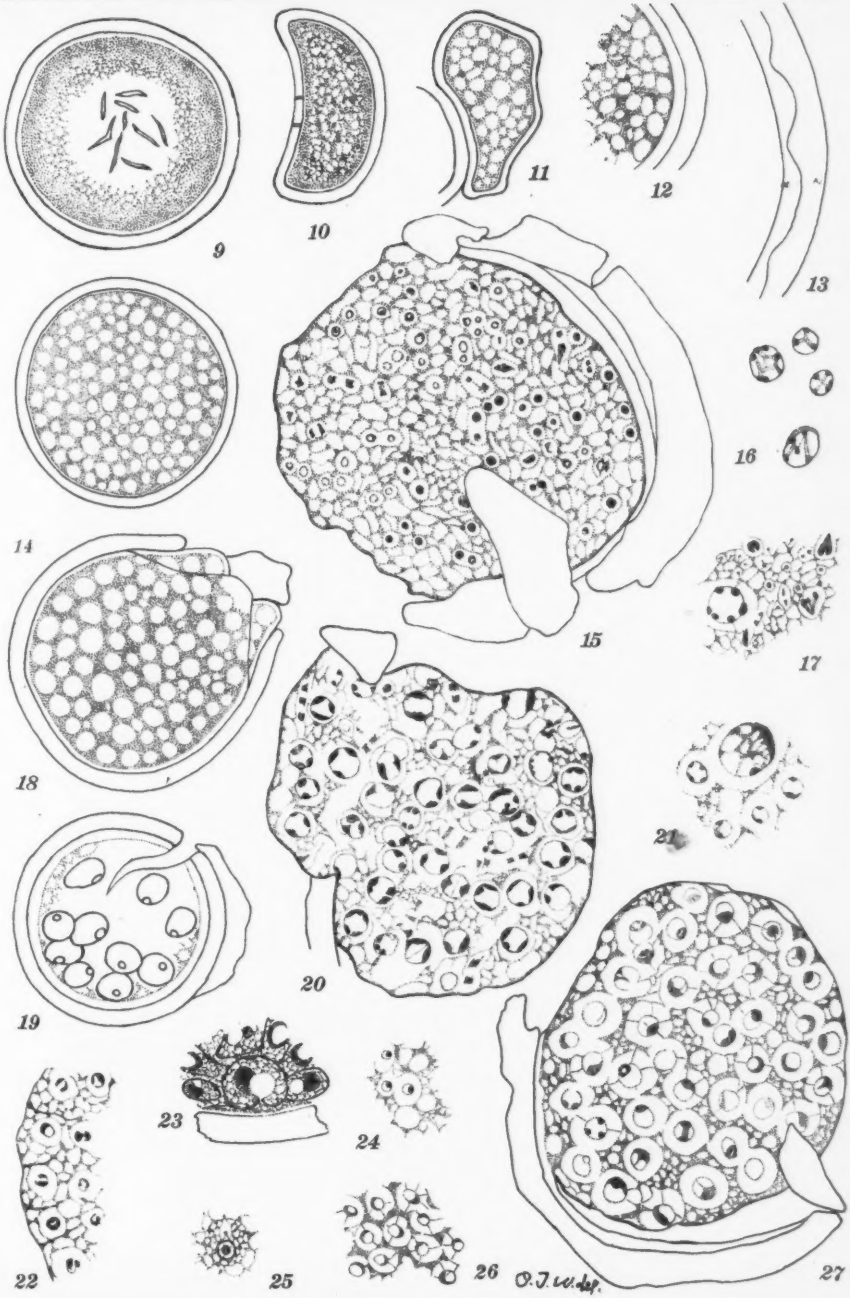
FIG. 51.—Budding or fragmentation of plasmodium; ×1800.

FIG. 52.—Nuclei of sporange just preceding formation of spores; ×1800.

FIGS. 54–57.—Division figures in plasmodium within host; ×1800.



WILSON on CROWN-GALL



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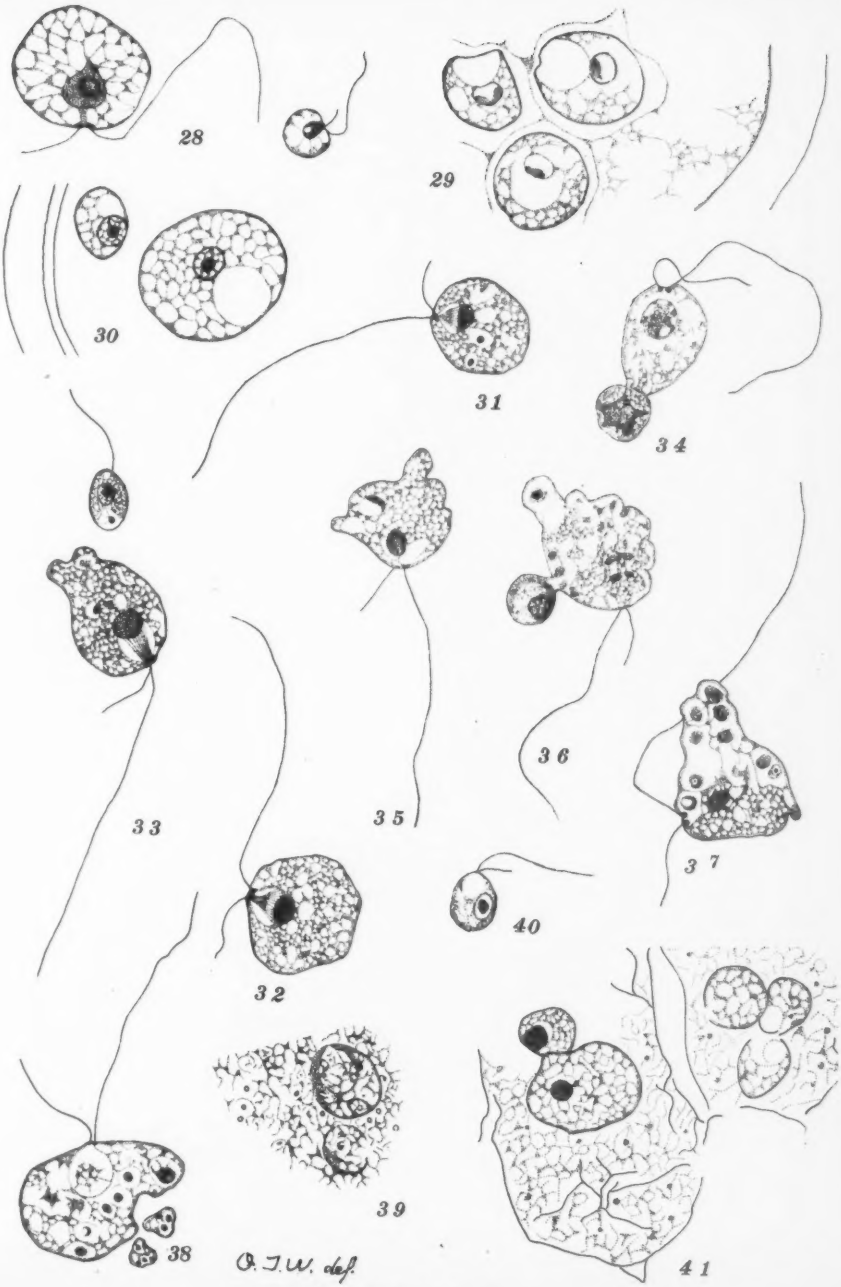
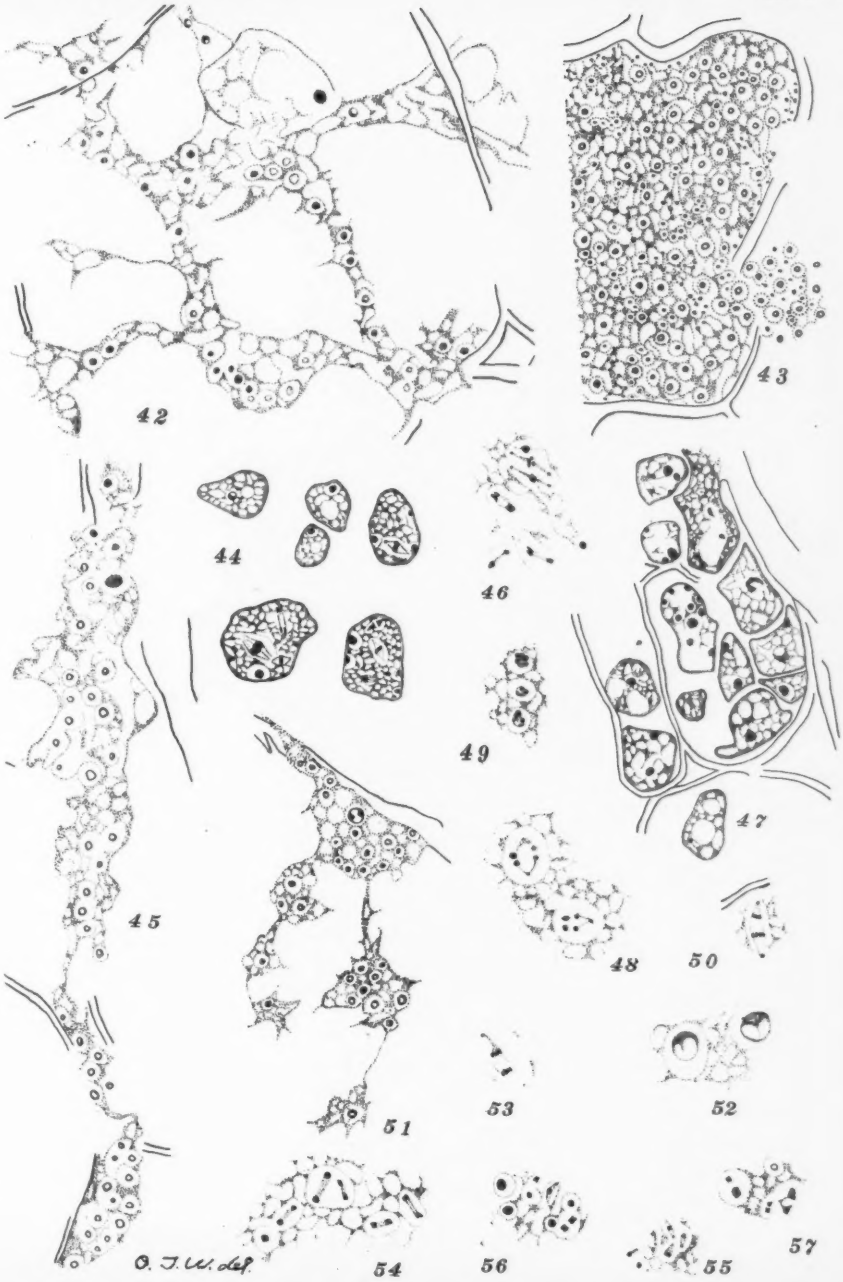


WILSON on CROWN-GALL



WILSON on CROWN-GALL

GEO-PRESENTATION AND GEO-REACTION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 268

EVA O. SCHLEY

(WITH FIVE FIGURES)

Historical

The reaction of plants to geotropic stimulation has been the subject of considerable investigation, the problem having been attacked from many standpoints. Naturally, perhaps, the physical side was studied first, a number of workers having developed the main features of gravity stimulus, presentation and reaction times, perception, conduction and response, organs of perception, and other related subjects. The chemical side of the field, involving the change in metabolism of the stimulated organ, has received much less attention.

The first worker in this field seems to have been KRAUS. As early as 1870 he published (**14**) the first of a series of researches on the chemical content of the growing plant, both in normal relations and after subjection to various external stimuli. This research included (1) the water content, (2) the acidity, (3) the sugar content of the normally growing shoot, (4) the relation of each to the growth maximum, and (5) steps in the change of the cell content of the concave and convex side of the geotropically and heliotropically responding organ. He determined that, in the normally growing shoot, (1) the acidity decreases from the tip downward, (2) the water increases relatively from the tip to the downward limit of growth, and (3) the sugar increases from the tip below the growth maximum and therefore is not a limiting factor in growth. In the stimulated organ he found on the convex-becoming side (1) an increase of sugar production up to the time of visible curvature and then a decrease, (2) a progressive decrease in acidity during stimulation, free acid being entirely absent from the responded organ, and (3) a progressive increase of water preceding curvature.

DEVRIES (5) studied the forces released in gravity stimulus, the effect of these released forces upon curvature, and the release of elasticity by gravity stimulus. He concludes that gravity produces an increase of osmotically active material in the cells of the convex-becoming side, causing an intake of water from the adjacent tissue, the resulting increased turgor producing a longitudinal extension of the elastic cell membranes, which, originally plastic, become fixed through growth and lignification.

CIELSIELSKI (2) observed a difference of the cell sap on opposite sides of geotropically stimulated roots, the cells of the convex-becoming flank exhibiting a thin watery protoplasm in contrast with the denser, more opaque plasma of the side becoming concave. KOHL (13) obtained analogous results in the sporangiophores of *Phycomyces*, in that in geotropic stimulation the plasma of the concave side of the filament became much thicker, while that of the convex side became thin and watery. He concluded that there was a causal relation between this differentiation of cell plasma and the curvature of the organ. ELFVING (7), however, according to his reviewers, produced a similar differentiation of protoplasm in *Phycomyces* sporangiophores by allowing them to push against a glass obstruction, a purely mechanical stimulation.

HILBERG (11), contrary to DEVRIES' results, found that in geotropic stimulation the osmotic pressure of the concave side of leaf joints and stem nodes of various plants is greater than that of the convex side. WORTMAN (22) negatives both DEVRIES' and HILBERG'S conclusions, since he could find no difference in the osmotic pressure of the two flanks of stimulated organs, and holds DEVRIES' view of the causal relation between turgor and curvature to be wholly untenable. On the other hand, he agrees with KOHL in that he found in geotropically stimulated organs the plasma "wandered" from the convex to the concave side, the thickened plasma inducing the cell membranes of the concave side to become thicker but less elastic and less extensible than those of the convex side. These latter, stretching longitudinally, force the concave side upward, thus producing curvature. NOLL (16) confirms WORTMAN'S work, but refutes his argument of the causal relation between the changed activity of the plasma and the curvature of the organ.

COPELAND (3) was unable to detect a difference in the opposite flanks of stems split lengthwise and stimulated geotropically four days. KERSTAN (12), using the plasmolysis method, found no increase of turgor in either flank of geotropically or heliotropically stimulated shoots either during curvature or after its completion. He concludes with NOLL that the decrease of turgor is due to the fact that the osmotic producing substances do not keep pace with the intake of water of the cells and their increased volume.

THATE (20) found KRAUS's method too crude to determine the difference of water in the two flanks of heliotropically stimulated shoots, although he does not dispute its existence. On the other hand, TONDERA (21) was able to verify KRAUS on this point and from his studies developed the law: "As the cells of the rind parenchyma of the lower organ half become filled by the streaming of water, due to gravity, the cells of the opposite half become water-poor, the resulting difference in pressure forcing the organ to move toward the water-poor half." This at best is a very crude conception.

From the cytological standpoint, MCDOUGAL (15) found that the cells of the convex side are greater in length, breadth, and thickness than those of the corresponding tissue of the concave side of geotropically stimulated roots. GEORGEVITCH (8) confirms this earlier work, while BÜCHNER (1) found the same condition in shoots that had been prevented from responding to gravity stimulation.

CZAPEK (4) is probably the chief worker in the chemical field of geo-presentation and reaction. Working with normal seedlings, he found that homogentisic acid is produced as a product of the oxidation of tyrosin, through the action of an oxidase, tyrosinase. In geotropic stimulation the tyrosin is converted into homogentisic acid by tyrosinase, as in normal seedlings, but the further oxidation of the homogentisic acid by the oxidase is inhibited by the production of an anti-oxidase, which renders the oxidase partly ineffective and by this means causes an accumulation of homogentisic acid. The accumulation begins after five minutes' stimulation, reaches a maximum at the time of distinct curvature, and disappears when reaction is complete. GROTTIAN (10) and GRAFE and LINSBAUER (9), however, were unable to confirm his results. They found as

great variation in the amount of homogentisic acid in different analyses of normal shoots as CZAPEK found between the stimulated and unstimulated organs.

In her work on thermotropism of roots, ECKERSON (**6**) found the greater permeability to be on the concave side of the root, and that this permeability changed with the changed thermotropic reaction of the root.

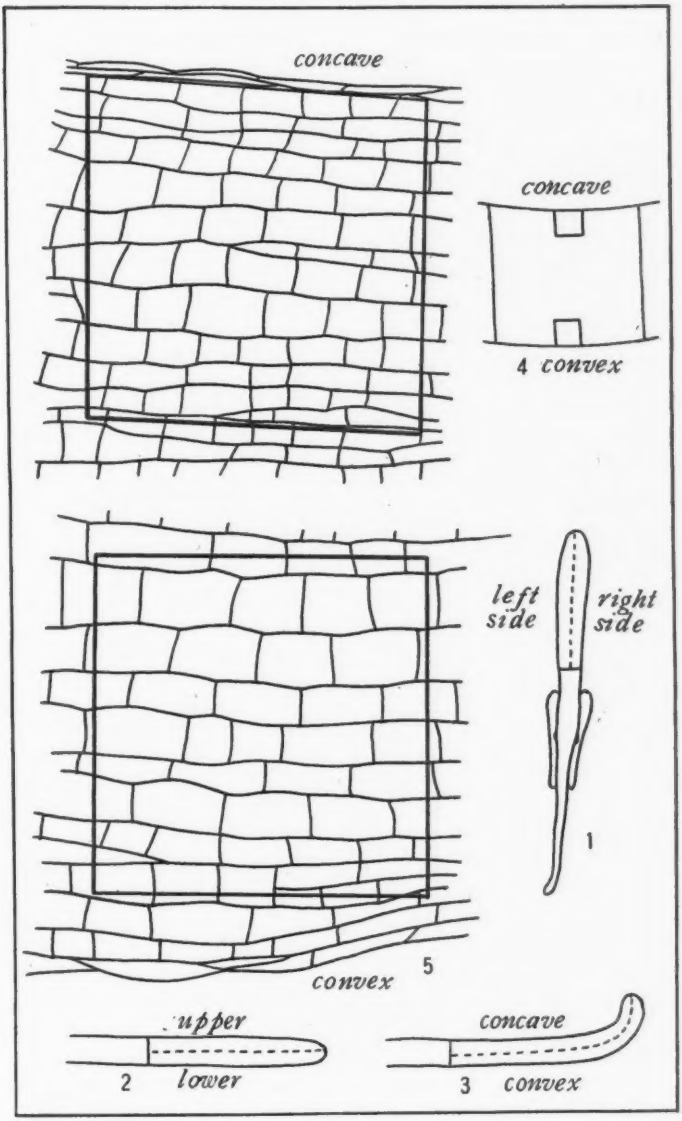
Scope of experiment

The work here presented is a continuation of that reported in a previous paper (**18**), which dealt with the acidity of the normal shoot, and compared the acidity of the two flanks of the geotropically stimulated shoot. The present paper deals with the changes in metabolism of the carbohydrates, the difference in osmotic pressure, and the difference in respiration of the upper and lower flanks of the geotropically stimulated shoot through presentation and reaction periods. *Vicia Faba* seedlings were employed throughout the experiment because they respond readily to geotropic stimulation, and because they are large enough to be easily split longitudinally. Trouble was experienced in germinating the seeds because of the development of mold. To overcome the difficulty the seeds were washed in tap water and then soaked two or three minutes in a 1 per cent solution of silver nitrate and rinsed thoroughly. They were grown in sand which had been sterilized by boiling in water an hour or longer, and then put into sterile pots while hot and allowed to stand until the following day before planting the seeds.

Carbohydrates and proteins

For this analysis etiolated seedlings of *Vicia Faba*, grown in sand in the greenhouse at a temperature of about 20° C., were used. When the seedlings were 6–8 cm. high they were geotropically stimulated for periods ranging from 15 minutes to 5 hours. Duplicate analyses of samples for each period of stimulation, as well as duplicate analyses of the unstimulated organ as controls, were made.

The epicotyls were split longitudinally into right and left halves (fig. 1) in the controls, and into upper and lower halves


concave
concave
4 convex
left side
right side
1
convex
5
upper
2
lower
concave
3
convex

FIGS. 1–5

(fig. 2) in the stimulated seedlings. These latter become the concave and convex halves (fig. 3) respectively in the responded organ. Samples varying from 6 to 10 gm. were used. The fresh portions were weighed in weighing bottles and the weight obtained by difference. The tissue, thus obtained, was cut up fine and triturated, killed in boiling 85 per cent alcohol, and boiled for 30 minutes.

The triturated tissue was subjected to alcoholic extraction for 3 hours and to ether extraction for 2 hours in the Koch modification of the Soxlet extractor. The tissue was then pulverized and extracted in boiling water 30 minutes. This water extraction was repeated six times. Following this was another alcohol extraction of 24 hours. The original killing alcohol, the ether extract (the ether was evaporated and the extract brought into solution in water), the water extract, and the two alcohol extracts were combined, and the volume increased to 500 cc. by the addition of water. This extract contained all the material soluble in these solvents, that is, the sugars, the lipoids, and the amino acids, and may be designated F_1. The residue contained the insoluble substances (starches, pectins, hemicelluloses, and cellulose), and may be designated F_2. From F_1 was taken three 150 cc. portions for the determination of (1) sugar, (2) nitrogen, and (3) dry weight. From F_2 was obtained (1) the dry weight and (2) the hydrolyzable polysaccharides.

The alcohol-water-soluble portion for the determination of sugar was freed from alcohol by evaporation on the steam bath, water being added before and during the process of evaporation, and the final volume brought to 150 cc. The tannins and lipoids were precipitated by the addition of a 10 per cent solution of basic lead acetate, the volume made up to 200 cc. and filtered immediately. The excess lead was precipitated from 150 cc. of the filtered solution by means of a saturated solution of ammonium sulphate, and the volume brought again to 200 cc. Duplicate determinations of 50 cc. portions were made of sugar solutions thus obtained, by the Munson and Walker method for the determination of reducing sugars (**17**). The amount of cuprous oxide thus obtained was determined by the volumetric potassium permanganate

method (*op. cit.*, pp. 52, 53.). An N/20 solution of permanganate was used. A third 50 cc. portion of this clarified solution was used for the determination of non-reducing or hydrolyzable sugars. The hydrolysis was performed according to the method for the determination of sucrose in the absence of raffinose (*op. cit.*, pp. 40, 41). The cooled solution was neutralized with 20 per cent sodium hydrate, brought to 100 cc. volume, and duplicate sugar estimations made of 50 cc. portions as described.

The residue (F_2), after the determination of the dry weight (to be described later), was used for the determination of the polysaccharides according to the method for direct acid hydrolysis of starch (*op. cit.*, p. 53). Duplicate determinations of 50 cc. portions were used for the determination of sugars, as in F_1.

The calculations were based on the milligrams of copper oxidized in the change from cuprous to cupric oxide, and expressed in equivalent milligrams of dextrose obtained from the Munson and Walker table accompanying the method of analysis (p. 243).

The portion of F_1 for the determination of dry weight was evaporated to moist dryness on the steam bath and brought to constant weight in vacuo. The dry weight of F_2 was obtained by bringing the residue of the original tissue to constant weight in the electric oven at a temperature of 104° C. The calculations for both were based on the dry weight per gram of the fresh material.

The third portion of F_1 was used for the determination of the total nitrogen. This determination was made after the Kjeldahl method as modified by ARNOLD. Calculations were made on the amount of nitrogen per gram of fresh weight. Table I shows the results obtained. It will be noticed that the soluble sugars vary but little throughout. The hydrolyzable sugars increase markedly at the time of visible response, and are greater on the convex side. The polysaccharides decrease as the hydrolyzable sugars increase. The dry weight of F_1 remains practically constant. The dry weight of F_2 remains practically constant until the beginning of curvature, when the weight of the convex side becomes less. The results of this sugar determination are not comparable with the work of KRAUS, for he was working with the raw pressed sap, which probably contained reducing substances other than sugars, as he himself suggests.

KRAUS found that the reducing substances increased on the convex side of the responding organ up to the time of visible curvature, and then decreased on that side of the curved shoot, a point upon

TABLE I

DETERMINATION OF MATERIAL PER GRAM FRESH WEIGHT

TIME STIMULATED	F_1 Soluble sugar (mg.)	F_1 Hydrolyzable sugar (mg.)	F_2 Polysaccharide (mg.)	F_1 Total carbohydrates (mg.)	F_2 Dry weight (g.)	F_3 Dry weight (g.)	F_1 Total nitrogen (g.)
Sample I, unstimulated							
8.74 (right)	23.45	3.21	3.60	30.26	0.06845	0.01920	0.004800
9.35 (left)	23.30	3.95	3.31	30.56	0.06878	0.01876	0.004535
Sample II, stimulated 15 min.							
11.88 (upper)	23.58	2.96	5.75	32.29	0.07124	0.02449	Lost
12.05 (lower)	22.20	3.10	4.93	30.23	0.06894	0.02176	Lost
Sample III, stimulated 30 min.							
10.38 (upper)	19.45	3.54	3.37	26.36	0.06460	0.01809	Lost
10.38 (lower)	19.35	3.58	3.02	25.95	0.06888	0.01887	Lost
Sample IV, stimulated 1 hour							
11.58 (upper)	22.44	4.94	4.00	31.38	0.06296	0.01994	0.004346
12.20 (lower)	22.00	4.73	2.98	29.71	0.06621	0.02014	0.004353
Sample V, stimulated 1.5 hours							
7.17 (upper)	21.90	6.34	4.72	32.96	0.05788	0.02224	0.004238
7.09 (lower)	21.62	4.82	4.66	31.10	0.06379	0.02146	0.004448
	Beginning visible response						
Sample VI, stimulated 2 hours							
10.23 (upper)	18.65	6.20	2.44	27.29	0.05803	0.01828	0.003697
10.15 (lower)	21.50	9.15	3.66	34.31	0.06318	0.01832	0.003838
Sample VII, stimulated 3 hours							
9.32 (upper)	23.10	8.34	3.29	34.73	0.06716	0.02054	0.004421
9.05 (lower)	28.25	9.10	3.50	40.85	0.06802	0.01950	0.004738
Sample VIII, stimulated 4 hours							
9.69 (upper)	23.05	4.90	3.44	31.39	0.07426	0.02100	0.003985
9.66 (lower)	23.45	7.86	3.18	34.49	Lost	0.01875	0.004144
Sample IX, stimulated 5 hours							
6.92 (upper)	21.10	9.20	1.88	32.18	0.06713	0.01907	0.004855
7.63 (lower)	26.75	13.82	2.62	43.19	0.06783	0.01686	0.004593

which he has frequently been misquoted (**14**, pp. 87 and 89). The total nitrogen remains constant throughout, the unstimulated sample I showing almost identically the value of sample IX at the close of the experiment.

Osmotic pressure

The osmotic pressure of the two flanks during the period of presentation and response was determined by means of plasmolysis. Weight molecular solutions of cane sugar and potassium nitrate were used as plasmolyzing agents. The seedlings were geotropically stimulated for varying periods of time. Portions of the seedlings, including the region of response, were sectioned (on the hand microtome) vertically, that is, from upper to lower side of the horizontally placed shoot. The sections were placed in weight molecular solutions of the plasmolyzing agent of such percentages as previous experiment had shown to be close to the plasmolyzing point. The series of weight molecular solutions was graduated to intervals of one-half of 1 per cent. The accompanying tables and graphs show the results of one each of the experiments made.

PLASMOLYSIS

(Using cane sugar as plasmolyzing agent)

In normal shoots both sides plasmolyze at 42 per cent weight molecular
After 5 minutes' stimulation both sides plasmolyze at 42 per cent
After 10 minutes' stimulation both sides plasmolyze at 43 per cent
After 45 minutes' stimulation upper side at 43, lower side at 44 per cent
After 1.5 hours' stimulation upper side at 43, lower side at 44.5 per cent
After 5 hours' stimulation both sides at 43 per cent

(Using potassium nitrate as plasmolyzing agent)

In normal shoots both sides plasmolyze at 31 per cent weight molecular
Stimulated 15 minutes both sides plasmolyze at 31 per cent
Stimulated 30 minutes both sides plasmolyze at 32 per cent, upper general, lower a few cells
Stimulated 45 minutes upper faintly at 33, lower at 33.5 per cent
Stimulated 1.25 hours upper at 32, lower at 33 per cent
Stimulated 5 hours both sides at 32 per cent

These results indicate that the osmotic pressure of the cell rises as the time of stimulation increases, reaches a maximum at or before visible response, and decreases as the response nears completion. The osmotic pressure is greater on the convex side during the period of response. It is interesting to note that the maximum acidity, as shown in a previous paper (18), is reached in 30 minutes, while the maximum turgor is reached in 45 minutes. The results of different investigators on the turgor change of stimulated shoots

show little agreement. Some writers have found the greater turgor on the concave, some on the convex, side; others have found no difference in turgor in either flank of the stimulated shoot or in the stimulated versus the unstimulated organ, with the balance of the argument rather in favor of the last mentioned. Inspection of the work of those writers who have tabulated their results, however, shows that the time of stimulation (ranging in general from several hours to several days) was too long to catch the change in turgor, which change appears to take place in a relatively short time, as was originally determined by DEVRIES in his macroscopic turgor experiments on geotropically stimulated grass nodes.

Respiration

Qualitative experiments were conducted upon the relative respiration of stimulated and unstimulated roots, and upon the upper and under flanks of geotropically stimulated shoots. These experiments were made in the TASHIRO (**19**) biometer apparatus, which determines the relative rate of respiration by the precipitation of barium carbonate on the surface of a drop of barium hydrate in a closed chamber.

The roots, without previous stimulation, were placed, one horizontally and one vertically, in similar chambers designated as left and right respectively. The shoots were stimulated for periods varying from 10 minutes to 5 hours. They were split longitudinally just before being placed in the apparatus. The roots were suspended and the shoots were placed horizontally, the upper with the cut surface down, and the lower with the cut surface up, as during stimulation. Both were placed across Van Tieghem cells in order to give equal opportunity for carbon dioxide diffusion. Many seedlings were tested with uniform results.

Table II shows that a geotropically stimulated root has a higher rate of respiration than the unstimulated root, and that in the stimulated shoot the under (convex) side shows a higher rate of respiration than the upper (concave) side at all intervals of time during stimulation and response that were investigated. It also shows that the rate of respiration decreases as the time of stimulation increases.

The effect of geotropic stimulation upon the cell structure of the responded shoot was determined through microscopical examination. Longitudinal sections from concave to convex side of the completely responded shoot in the region of the angle of greatest curvature were cut on the freezing microtome, and camera lucida drawings were made of corresponding areas on the concave and convex flanks of the organ (figs. 4, 5).

TABLE II

RELATIVE RESPIRATION

Seedling	Left chamber	Right chamber	Time stimulated	Time in apparatus	Greater precipitation of $BaCO_3$
Roots					
Sunflower...	Horizontally placed	Vertically placed	.....	4 minutes	Horizontal root
Sunflower...	Vertically placed	Horizontally placed	.....	4 minutes	Horizontal root
Zea Mays...	Horizontally placed	Vertically placed	.....	4 minutes	Horizontal root
Shoots					
Vicia Faba..	Convex side	Concave side	10 minutes	2 minutes	Convex (much greater)
Vicia Faba..	Convex side	Concave side	2 hours, 31 minutes	3 minutes	Convex side
Vicia Faba..	Concave side	Convex side	4 hours, 58 minutes	7 minutes	Convex side

A study of fig. 5 shows that the cells on the convex side are larger than those on the concave side. A 10 cm. square on the convex side contains 40 cells, while a corresponding area on the concave side shows 72 cells. This result is in accord with the work of previous investigators.

Summary

1. The reducing sugars remain constant throughout stimulation and response.

2. The hydrolyzable sugars increase on the convex side at the expense of the polysaccharides as response takes place.

3. The total sugars are constant until beginning of response, when the sugars of the convex side become greater.

4. The osmotic pressure increases until visible curvature has taken place. At the end of the reaction both flanks show the same osmotic pressure, which, however, is greater than that of the normal shoot.

5. Respiration of the geotropically stimulated root is greater than that of the unstimulated organ.

6. The rate of respiration of the convex side of the geotropically stimulated shoot is greater than that of the concave side throughout the period of perception and response.

7. Respiration decreases as the time of stimulation increases.

8. The steps, in point of time, of the chemical changes that take place in a geotropically stimulated shoot are: (1) increased respiration, (2) increased acidity (18), (3) increased turgor, and (4) increased production of hydrolyzable sugars with corresponding decrease of polysaccharides on the convex side of the responding organ.

The writer is greatly indebted to Dr. WILLIAM CROCKER, who suggested the problem, and who gave much assistance during the progress of the work; to Dr. F. C. KOCH for help in the methods of analysis; and to Dr. SHIRO TASHIRO for assistance in the work on respiration.

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BRIEFER ARTICLES

SECTIONING HARD WOODY TISSUES

In preparing hard or refractory woods for sectioning it has been customary to soften in hydrofluoric acid and imbed in either celloidin or gelatin, the latter process being favored in the sectioning of partly disorganized material. In connection with certain anatomical problems, a method of imbedding in paraffin after the demineralization of the woody tissues by means of hydrofluoric acid has been developed, and has proved most successful in dealing with either hard woody tissues or non-homogeneous objects, possessing both soft delicate tissues and hard lignified structures. More uniform results are assured with this method, and it is also possible thus to secure an unbroken series of sections without the tedious and complicated process involved in the celloidin method.

PREPARATION OF MATERIAL.—The woody material is first cut into blocks of a convenient size for sectioning. In the case of hard stems and roots a fine sharp hacksaw should be used to prevent the tearing or jamming of the tissues in the vicinity of the cut surfaces. More delicate material, such as small roots, seedlings, herbaceous stems, etc., may be cut into smaller portions by means of a sharp knife, preferably a Gillette razor blade. If the material to be examined is dead and dry, it should be repeatedly boiled in water and cooled to remove all air from the tissues, as in the celloidin method. If living, it should be well fixed in some suitable reagent. A mixture of formalin, alcohol, and glacial acetic acid has proven satisfactory for most anatomical work: 50 per cent alcohol, 100 cc.; formalin (commercial), 6 cc.; glacial acetic acid, 3 cc. Fix 24–48 hours and rinse thoroughly in running water.

DEMINERALIZATION.—The blocks of material thus prepared are ready for the next step in the process, which is the demineralization and general softening of the lignified structures of the stem by means of hydrofluoric acid. The blocks are transferred directly from water to either 50 per cent aqueous solution of commercial hydrofluoric acid or hydrofluoric acid full strength. The strength of the acid and the length of time in the reagent depend, of course, upon the nature of the material. Cubes of very hard woods of a comparatively homogeneous structure, such as the oak, require 3–4 weeks in 50 per cent hydrofluoric

acid. Pure acid may be used if it is desired to hurry the process, but great care should be taken and the material should be examined often and removed as soon as it cuts easily with a Gillette razor blade. Rhizomes of *Osmunda* and similar material, possessing very hard sheathing leaf bases, may remain in 50 per cent acid several months without apparent injury to other than the most fragile parenchyma tissues. Blocks of *Dioon spinulosum* 1–2 cm. square were treated with 50 per cent acid 3–6 weeks with gratifying results. *Welwitschia*, the delicate parenchyma tissue of which is crowded with rigid spicular cells of great size, after treating with 50 per cent hydrofluoric acid and imbedding in paraffin, can be sectioned without difficulty. *Rhipogonum scandens*, a New Zealand liana, impossible to section by ordinary methods due to the extensive amount of sclerenchymatous tissue distributed through the stem, especially surrounding the scattered bundles, sectioned with perfect ease after immersion for one week in full strength hydrofluoric acid. Non-homogeneous material, such as corn stem, usually difficult to section, especially after it attains a diameter of 1.5–2.5 cm., because of the rigidity of the bundles and the delicate character of the parenchyma, was treated with a 25 per cent solution of hydrofluoric acid for one week, and sections 15–20 μ in thickness were easily cut from 52° C. paraffin. In order to minimize the time of heating of such material in the paraffin bath during the infiltration process, blocks should not average more than 1–1.5 cm. in thickness. The leaves and stem of wheat, oats, and other cereals also contain more or less silica, which makes them very refractory objects to cut, and accounts for the difficulty in obtaining sections of uredospores and teleutospores of *Puccinia graminis*. Immersion of these leaves in 10 per cent solution of hydrofluoric acid for a few days, possibly a week, should remove much of the silica without appreciable injury either to the cell walls or cell contents. After removal from the softening medium, the material should be thoroughly washed in running water to remove all traces of the reagent and then placed in 60 per cent alcohol.

Dehydration and clearing.—In passing through the alcohol and xylol series in the process of dehydration and clearing, the time required for each stage ranges from 12 hours for each of the 60, 70, 80, and 95 grades of alcohol, to 24 hours for the absolute alcohol and each of the absolute alcohol and xylol series. Four grades, 25, 50, 75, and 100 per cent xylol, are generally sufficient. Any air or gases remaining in the tissues should be removed by means of a vacuum pump while the woody material is in pure xylol before the addition of the paraffin.

INFILTRATION WITH PARAFFIN.—During the first 36 hours in the process of infiltration with paraffin the wood is kept *on* the paraffin bath, but shortly before the mixture of xylol and paraffin is replaced with pure melted paraffin; both the material and the paraffin mixture are transferred to a flat dish of some kind to facilitate a quick evaporation of the xylol and then placed *in* the bath. At least two or three changes of paraffin are usually desirable. Special care has to be taken at this point, the best results being obtained when such woody or partially woody material is carried through the final process of infiltration with paraffin (melting point 52° C.) from 48 to 72 hours.

SECTIONING.—With a proper allowance of time for infiltration, sections of the most refractory tissues ranging from 10 to 30 μ in thickness may be cut with a sliding microtome with perfect ease, and a complete series obtained by removing each section, as cut, from the knife and placing it directly upon a slide well coated with albumen fixative and flooded with water. All paraffin sections thus cut and not held in ribbon are likely to curl. To prevent this curling of the section as it comes upon the knife it has been the writer's practice, after flooding the surface of the object and the knife with water (using ice water in warm weather and slightly warmed water in cold weather), to hold a camel's hair brush or preferably the tip of the first finger lightly against the section as it is being cut. The section, unless of considerable size, will then adhere to the moist finger tip and can thus be transferred to the slide without danger of tearing or crushing. With practice sections may be cut and transferred from the microtome knife to the slide very rapidly by this method, and the problem of curling entirely obviated.

Subsequent stages in the fixing of sections to the slide, removal of paraffin, staining and mounting, follow the usual paraffin schedule.—LADEMA M. LANGDON, *University of Chicago.*

CAMPHORINA VS. CINNAMOMUM

In a short article on the botanical nomenclature of the Pharmacopoeia, FARWELL[1] proposes to adopt the generic name *Camphorina* Noronha (1790) in place of *Cinnamomum* Blume (1825), although the latter, originally proposed by TOURNEFORT, had been used by LINNAEUS in the first edition of his *Systema* in 1735. It is not my object to discuss the validity of this proposed change, but aside from calling attention

[1] The Druggists' Circular **62**:535. 1918. The first paper of the series was published in Botanical Nomenclature of the U.S.P. IX, *op. cit.* **61**:173–176. 1917.

to the fact that a considerable number of new names have been published in a strictly trade journal where they will probably not be noticed by systematists, attention should be called to the naïve and wholly unnecessary publication of the binomial *Camphorina saigonica* Farwell, a *nomen nudum*, as follows: "The Saigon Cinnamon. *Camphorina Saigonica*, n.sp. The plant producing the Saigon Cinnamon has not as yet been definitely determined, but it is generally supposed to be an undescribed species. The bark is well described in the U.S.P. on pages 114 and 115, and I tentatively propose the above name for the species producing it." In the opening statement it appears as *Cinnamomum saigonicum*, which may also be credited to FARWELL as a *nomen nudum*, although this is the name of the drug used in the 1905–1907 and 1916 editions of the U.S. Pharmacopoeia, where the comment is added: "The bark of an undetermined species of *Cinnamomum*." KRAEMER[2] states that Saigon cinnamon is obtained from *Cinnamomum Loureiri* (?) and other species cultivated in Cochin China and parts of China and exported from Saigon, so that it would appear that the species yielding the product is by no means generally assumed to be an undescribed species as FARWELL indicates.

Knowing from experience the great difficulty of identifying species of *Cinnamomum*, even when complete material is available, I communicated FARWELL'S proposition to Dr. A. CHEVALIER, Director of the Institut Scientifique in Saigon, the following quotation being from his letter of July 21, 1919: "Je vous remercie de m'avoir communiqué un renseignement bibliographique sur la cannelle de Saigon. Il n'existe pas dans le commerce de cannelle de Saigon. Celle qui est exportée par le port de Saigon a été achetée par les marchands chinois en Annam ou elle est fournie par le *Cinnamomum Loureiri* Nees." See also A. CHEVALIER in Bull. Écon., Indochine **22**:526. 1919.

Although *Cinnamomum Loureiri* is not admitted by LECOMTE[3] as an Indo-Chinese species, CHEVALIER is doubtless correct in his identification. From the very fact that 7 species of *Cinnamomum* are definitely known from southern China and that 11 are known from Indo-China, coupled with the fact that the accessible parts of both regions are fairly well explored from a botanical standpoint, it is unreasonable to assume that a commercially important species such as the one under consideration has escaped detection up to the present time.

[2] Botany and pharmacognosy, p. 513. 1910; Scientific and applied pharmacognosy, p. 304. 1915.

[3] Fl. Gén. Indo-Chine **5**:109–117. 1915.

Exception may well be taken to the proposed new names under KAVA,[4] *Piper esculentum* Farwell, *Methysticum methysticum* Farwell, and *Methysticum esculentum* Farwell. These are proposed because *Piper methysticum* Forst. f. (1786) is assumed to be different from *P. methysticum* Linn. f. (1781). Before adopting the new names proposed by FARWELL it would be well to compare the actual types in London, as such comparison will probably show *Piper methysticum* Linn. f. and *P. methysticum* Forst. f. to be identical and based on material of the same (Forster's) collection. At any rate it would seem to be wholly unnecessary to publish *Piper esculentum* Farwell and *Methysticum esculentum* Farwell for the same species in the same article merely because there is a considerable difference of opinion among botanists as to the generic status of the plant in question. A taxonomist should be able to determine to his own satisfaction the status of a proposed genus before making transfers to it.—E. D. MERRILL, *Bureau of Science, Manila, P.I.*

[4] FARWELL, O. A., Botanical nomenclature of the N[ational] F[ormulary] IV, *op. cit.* **61**:229–232. 1917. There is a continuation of this paper, *op. cit.* **63**:49, 50. 1919.

CURRENT LITERATURE

NOTES FOR STUDENTS

Taxonomic notes.—ENGLER[1] has described a new genus (*Hieronymusia*) of Saxifragaceae from South America (Argentina and Bolivia). It was referred first to *Saxifraga* (*S. alchemilloides*) and later to *Suksdorfia*.

SCHLECHTER,[2] in continuation of his studies of orchids, has described 19 new species, representing 9 genera, chiefly collected in Argentina by DUSEN.

ST. JOHN[3] has described a new genus (*Phanerotaenia*) of Umbelliferae, based on *Polytaenia Nuttallii texana* C. and R., of Texas and Oklahoma.

SETCHELL and GARDNER[4] have begun the publication of the marine algae of the Pacific Coast, the first part including the blue-green algae. The group as presented contains 30 genera, representing 6 families. Of the 93 species included, 35 are described as new.—J. M. C.

Polycotylous seedlings.—Miss BEXON[5] has found that *Centranthus ruber* (Valerianceae) develops a remarkably high proportion of polycotylous seedlings. From two square yards of soil in which self-sown seeds of this species were germinating, 87 polycotylous specimens were obtained and studied. They are grouped as hemitricotyls, tricotyls, hemitetracotyls, and tetracotyls, terms which are self-explanatory. The vascular anatomy of these various conditions was investigated, with some interesting results. A "twinned" seedling was of special interest, the twinning being due either to the fusion of two distinct embryos, or to the partial separation of the product of the embryo initial.—J. M. C.

Unusual monocotyledonous roots.—Miss SPRATT[6] has discovered some interesting anomalies in the roots of some monocotyledons. In *Dracaena* there

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[1] ENGLER, A., *Hieronymusia* Engl., eine neue Gattung der Saxifragaceen. Notizbl. Königl. Bot. Gart. **7**:265–267. *fig. 1.* 1918.

[2] SCHLECHTER, R., III. Orchidaceae novae, in caldariis Horti Dahlemensis cultae. Notizbl. Königl. Bot. Gart. **7**:268–280. 1918.

[3] ST. JOHN, H., *Phanerotaenia*, a new genus of Umbelliferae. Rhodora **21**:181–183. 1919.

[4] SETCHELL, W. A., and GARDNER, N. L., The marine algae of the Pacific Coast of North America. I. Myxophyceae. Univ. Calif. Publ. Bot. **8**:1–138. *pls. 1–8.* 1919.

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are two kinds of secondary vascular development; in the one case the pericycle becomes meristematic and adds vascular elements to the stele; and in the case of large roots this is followed by a similar behavior of the cortical cells just outside the endodermis. In *Pandanus* and *Yucca* internal vascular strands were discovered, which are differentiated at the growing point. In the aerial roots of certain Araceae, inside of the radial primary vascular cylinder, there occur scattered groups of xylem and phloem vessels.—J. M. C.

Sex intergrades.—YAMPOLSKY[7] has brought together the evidence of sex intergrades in flowering plants. The discussion is based upon his results with *Mercurialis annua*, in which he had found sexuality to be a fluctuating character. At the close of the paper he gives a long list of families showing transition from the monoecious to the dioecious condition, and also tabulates the results under 12 types. The general conclusion confirms the view that the potentialities of both sexes exist in all plants, and are not localized in any particular region or cells.—J. M. C.

Endosperm development in Vaccinium.—STEVENS[8] has described an interesting case of endosperm development in *Vaccinium corymbosum*. Two distinct types of initial endosperm development recognized are free nuclear division and wall formation, and these types have been regarded as characteristic of different genera and even families. In *V. corymbosum*, from material collected from a single plant, STEVENS finds that endosperm development may begin either way. This emphasizes the fact that many of our morphological categories have been too rigidly defined.—J. M. C.

North American flora.—The second part of volume 24 chiefly consists of the completion of the genus *Parosela*, by RYDBERG, 43 species of which had been published in the preceding part.[9] This genus is recognized to include 178 North American species, 46 being described in the present contribution as new, and many being species transferred from other genera. The three other genera presented are *Thornbera* (13 species, 5 new), *Petalostemon* (42 species, 8 new), and *Kuhnistera* (2 species).—J. M. C.

New species of Piper.—In connection with the biological survey of Panama, conducted several years ago by the Smithsonian Institution, the collected material of the Piperaceae was sent to the late CASIMIR DECANDOLLE, whose determinations have just been published.[10] The collection was found to include 27 new species of *Piper* and 4 new varieties.—J. M. C.

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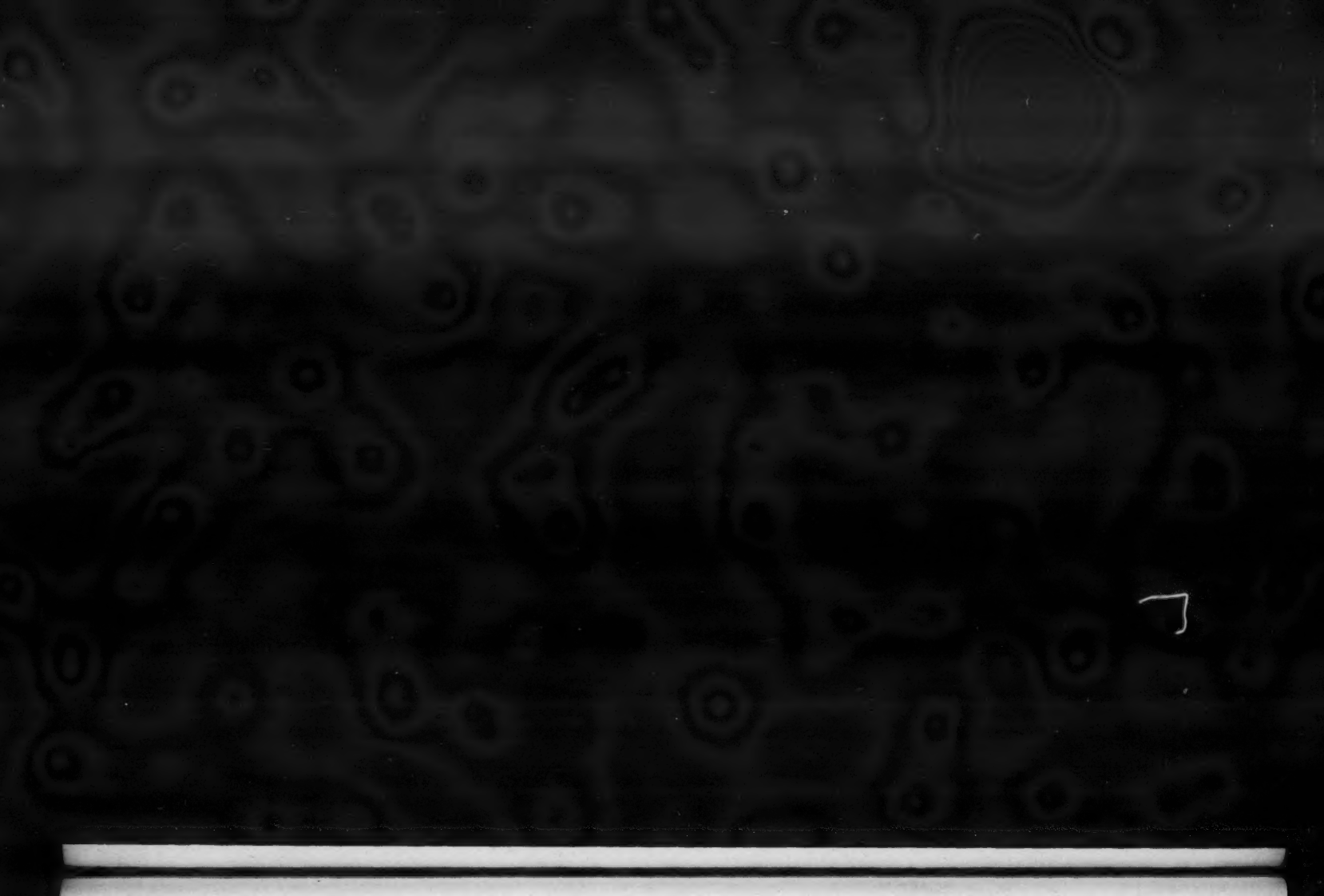
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